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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/US97/03794 <b>(22) International Filing Date:</b> 12 March 1997 (12.03.97) <b>(30) Priority Data:</b> 08/616,904 15 March 1996 (15.03.96) US <b>(71) Applicant:</b> UNIVERSITY OF FLORIDA [US/US]; 186 Grinter Hall, Gainesville, FL 32611 (US). <b>(72) Inventors:</b> SOOS, Jeanne, M.; 23 Hall Avenue, Watertown, MA 02172 (US). SCHIFFENBAUER, Joel; 368 N.W. 48th Boulevard, Gainesville, FL 32607 (US). JOHNSON, Howard, M.; 4404 N.W. 75th Street, Gainesville, FL 32606 (US). <b>(74) Agents:</b> SHOLTZ, Charles, K. et al.; Dehlinger & Associates, P.O. Box 60850, Palo Alto, CA 94306-0850 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> ORALLY-ADMINISTERED INTERFERON-TAU COMPOSITIONS AND METHODS  <b>(57) Abstract</b>  The present invention includes interferon-tau (IFN $\tau$ ) pharmaceutical compositions useful for oral administration to treat cancers, autoimmune disorders (particularly multiple sclerosis), cell proliferative disorders and viral disease.		

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ORALLY-ADMINISTERED INTERFERON-TAU  
COMPOSITIONS AND METHODS

Field of the Invention

The present invention relates to orally-administered pharmaceutical compositions containing  
5 interferon-tau and methods of uses thereof.

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### Background of the Invention

Conceptus membranes, or trophoctoderm, of various mammals produce biochemical signals  
 25 that allow for the establishment and maintenance of pregnancy (Bazer, *et al.*, 1983). One such  
 protein, ovine trophoblast protein-one (oTP-1), was identified as a low molecular weight protein  
 secreted by sheep conceptuses between days 10 and 21 of pregnancy (Wilson, *et al.*, 1979; Bazer,  
*et al.*, 1986). The protein oTP-1 was shown to inhibit uterine secretion of prostaglandin F<sub>2</sub>-alpha,  
 which causes the corpus luteum on the ovary to undergo physiological and endocrinological demise  
 30 in nonpregnant sheep (Bazer, *et al.*, 1986). Accordingly, oTP-1 has antiluteolytic biological  
 activity. The primary role of oTP-1 was assumed to be associated with the establishment of  
 pregnancy.

oTP-1 was subsequently found to (i) exhibit limited homology (50-70%) with interferon alphas (IFN $\alpha$ ) of various species (Imakawa, *et al.*, 1987), and (ii) bind to a Type I interferon receptor (Stewart, *et al.*, 1987). Despite some similarities with IFN $\alpha$ , oTP-1 has several features that distinguish it from IFN $\alpha$  including the following: oTP-1's role in reproductive biochemistry (other interferons are not known to have any role in the biochemical regulation of reproductive cycles), oTP-1's cellular source -- trophoblast cells (IFN $\alpha$  is derived from lymphocyte cells), oTP-1's size -- 172 amino acids (IFN $\alpha$  is typically about 166 amino acids), and oTP-1 is weakly inducible by viruses (IFN $\alpha$  is highly inducible by viruses). The International Interferon Society recognizes oTP-1 as belonging to an entirely new class of interferons which have been named  
10 interferon-tau (IFN $\tau$ ). The Greek letter  $\tau$  stands for trophoblast.

The interferons have been classified into two distinct groups: type I interferons, including IFN $\alpha$ , IFN $\beta$ , and IFN $\omega$  (also known as IFN $\alpha$ II); and type II interferons, represented by IFN $\gamma$  (reviewed by DeMaeyer, *et al.*, 1988). In humans, it is estimated that there are at least 17 IFN $\alpha$  non-allelic genes, at least about 2 or 3 IFN $\beta$  non-allelic genes, and a single IFN $\gamma$  gene.

15 IFN $\alpha$ 's have been shown to inhibit various types of cellular proliferation. IFN $\alpha$ 's are especially useful against hematologic malignancies such as hairy-cell leukemia (Quesada, *et al.*, 1984). Further, these proteins have also shown activity against multiple myeloma, chronic lymphocytic leukemia, low-grade lymphoma, Kaposi's sarcoma, chronic myelogenous leukemia, renal-cell carcinoma, urinary bladder tumors and ovarian cancers (Bonnem, *et al.*, 1984; Oldham,  
20 1985). The role of interferons and interferon receptors in the pathogenesis of certain autoimmune and inflammatory diseases has also been investigated (Benoit, *et al.*, 1993).

IFN $\alpha$ 's are also useful against various types of viral infections (Finter, *et al.*, 1991). Alpha interferons have shown activity against human papillomavirus infection, Hepatitis B, and Hepatitis C infections (Finter, *et al.*, 1991; Kashima, *et al.*, 1988; Dusheiko, *et al.*, 1986; Davis, *et al.*,  
25 1989).

In addition, type I interferons are useful in treating autoimmune diseases such as multiple sclerosis (MS). In fact, IFN $\beta$  has been tested and approved by the U.S. Food and Drug Administration (FDA) as an MS therapy.

Significantly, however, the usefulness of IFN $\alpha$ 's has been limited by their toxicity: use of  
30 interferons in the treatment of cancer, autoimmune disorders and viral disease has resulted in serious side effects, such as fever, chills, anorexia, weight loss, and fatigue (Pontzer, *et al.*, 1991; Oldham, 1985). These side effects often require (i) the interferon dosage to be reduced to levels that limit the effectiveness of treatment, or (ii) the removal of the patient from treatment. Such

toxicity has reduced the usefulness of these potent antiviral and antiproliferative proteins in the treatment of debilitating human and animal diseases.

The present invention provides methods of treatment for cancer, autoimmune diseases (such as MS) and for inhibiting cellular proliferation and viral infection. These methods do not have the toxic side effects associated with currently-used therapies, and employ a convenient route of administration.

#### Summary of the Invention

In one aspect, the present invention includes an improvement in a method of treating a disease condition in a mammal (*e.g.*, mouse, dog or human) responsive to treatment by interferon-tau (IFN $\tau$ ). The improvement comprises orally administering a therapeutically-effective amount of IFN $\tau$ . The orally-administered IFN $\tau$  is preferably ingested by the mammal. In a general embodiment, the IFN $\tau$  is orally-administered at a dosage of between about  $1 \times 10^5$  and about  $1 \times 10^8$  units per day, preferably at a dosage of between about  $1 \times 10^6$  and about  $1 \times 10^7$  units per day. The IFN $\tau$  may be, for example, ovine IFN $\tau$  (OvIFN $\tau$ ), *e.g.*, a polypeptide having the sequence represented as SEQ ID NO:2, or a human IFN $\tau$  (HuIFN $\tau$ ), *e.g.*, a polypeptide having the sequence represented as SEQ ID NO:4 or SEQ ID NO:6.

In one embodiment, the disease condition is an immune system disorder, such as an autoimmune disorder (*e.g.*, multiple sclerosis (MS), type I (insulin dependent) diabetes mellitus, lupus erythematosus, amyotrophic lateral sclerosis, Crohn's disease, rheumatoid arthritis, stomatitis, asthma, allergies or psoriasis). MS is particularly amenable to treatment using the methods of the present invention.

In another embodiment, the disease condition is a cell proliferation disorder, such as a cancer (*e.g.*, hairy cell leukemia, Kaposi's Sarcoma, chronic myelogenous leukemia, multiple myeloma, superficial bladder cancer, skin cancer (basal cell carcinoma and malignant melanoma), renal cell carcinoma, ovarian cancer, low grade lymphocytic and cutaneous T cell lymphoma, and glioma).

In yet another embodiment, the disease condition is a viral disease (*e.g.*, hepatitis A, hepatitis B, hepatitis C, non-A, non-B, non-C hepatitis, Epstein-Barr viral infection, HIV infection, herpes virus (EB, CML, herpes simplex), papilloma, poxvirus, picorna virus, adeno virus, rhino virus, HTLV I, HTLV II, and human rotavirus).

In another aspect, the invention includes a method of treating an autoimmune disorder in a subject (*e.g.*, a human subject), by orally administering a therapeutically-effective amount of interferon-tau (IFN $\tau$ ) to the subject. The orally-administered IFN $\tau$  is preferably ingested by the subject. Examples of autoimmune conditions amenable to treatment, dosages, and sources of IFN $\tau$  are as presented above.



The invention also includes a method of decreasing the severity or frequency of a relapse of multiple sclerosis (MS) in a human suffering from MS, by orally administering a therapeutically-effective amount of interferon-tau (IFN $\tau$ ) to the human. Examples of dosages and sources of IFN $\tau$  are as presented above.

5 In another aspect, the invention includes a method of treating a cell proliferation disorder in a subject (*e.g.*, a human subject), by orally administering a therapeutically-effective amount of interferon-tau (IFN $\tau$ ) to the subject. The orally-administered IFN $\tau$  is preferably ingested by the subject. Examples of cell proliferation disorders amenable to treatment, dosages, and sources of IFN $\tau$  are as presented above.

10 In still another aspect, the invention includes a method of treating a viral disease in a subject (*e.g.*, a human subject), by orally administering a therapeutically-effective amount of interferon-tau (IFN $\tau$ ) to the subject. The orally-administered IFN $\tau$  is preferably ingested by the subject. Examples of viral diseases amenable to treatment, dosages, and sources of IFN $\tau$  are as presented above.

15 A further aspect of the invention includes a method of enhancing fertility in a female mammal (*e.g.*, a human female), by orally administering a therapeutically-effective amount of interferon-tau (IFN $\tau$ ) to the mammal. Examples of dosages and sources of IFN $\tau$  are as presented above.

20 These and other objects and features of the invention will become more fully apparent when the following detailed description is read in conjunction with the accompanying drawings.

#### Brief Description of the Figures

Figure 1 shows the amount of OvIFN $\tau$  in NZW mouse sera after administration by either oral feeding (filled bars) or i.p. injection (open bars) as measured using an anti-viral assay.

25 Figures 2A, 2B and 2C show the prevention of chronic-relapsing experimental allergic encephalomyelitis (EAE) in SJL mice by orally-administered (Fig. 2C) and i.p.-injected (Fig. 2B) IFN $\tau$  as compared with mice receiving no treatment (Fig. 2A).

Figures 3A, 3B and 3C show sections of mouse spinal cord stained with cresyl violet for detection of lymphocyte infiltration from EAE-induced animals receiving either no IFN $\tau$  treatment (Fig. 3A), OvIFN $\tau$  treatment by i.p. injection (Fig. 3B) or OvIFN $\tau$  treatment by oral feeding (Fig. 30 3C).

Figure 4 shows induction of IL-10 by either single-dose or prolonged IFN $\tau$  treatment administered by i.p. injection or oral feeding.

Figure 5 shows relapses of EAE in SJL mice following removal of IFN $\tau$  treatment.

Figure 6 shows ELISA detection of anti-OvIFN $\gamma$  antibodies in the sera of OvIFN $\gamma$ -treated mice following i.p. injection or oral feeding of OvIFN $\gamma$ .

#### Brief Description of the Sequences

SEQ ID NO:1 is the nucleotide sequence of a synthetic gene encoding ovine interferon- $\gamma$  (OvIFN $\gamma$ ). Also shown is the encoded amino acid sequence.

SEQ ID NO:2 is an amino acid sequence of a mature OvIFN $\gamma$  protein.

SEQ ID NO:3 is a synthetic nucleotide sequence encoding a mature human interferon- $\gamma$  (HuIFN $\gamma$ ) protein.

SEQ ID NO:4 is an amino acid sequence for a mature HuIFN $\gamma$ 1 protein.

SEQ ID NO:5 is the nucleotide sequence, excluding leader sequence, of genomic DNA clone HuIFN $\gamma$ 3, a natural HuIFN $\gamma$  gene.

SEQ ID NO:6 is the predicted amino acid sequence of a mature human IFN $\gamma$  protein encoded by HuIFN $\gamma$ 3, encoded by the sequence represented as SEQ ID NO:5.

#### Detailed Description of the Invention

##### I. Definitions

*Interferon- $\gamma$*  refers to any one of a family of interferon proteins having at least one characteristic from each of the following two groups of characteristics: (i) (a) anti-luteolytic properties, (b) anti-viral properties, (c) anti-cellular proliferation properties; and (ii) about 45 to 68% amino acid homology with  $\alpha$ -Interferons and greater than 70% amino acid homology to known IFN $\gamma$  sequences (*e.g.*, Ott, *et al.*, 1991; Helmer, *et al.*, 1987; Imakawa, *et al.*, 1989; Whaley, *et al.*, 1994; Bazer, *et al.*, 1994). Amino acid homology can be determined using, for example, the LALIGN program with default parameters. This program is found in the FASTA version 1.7 suite of sequence comparison programs (Pearson and Lipman, 1988; Pearson, 1990; program available from William R. Pearson, Department of Biological Chemistry, Box 440, Jordan Hall, Charlottesville, VA). IFN $\gamma$  can be obtained from a number of sources including cows, sheep, ox, and humans.

An *interferon- $\gamma$  polypeptide* is a polypeptide having between about 15 and 172 amino acids derived from an interferon- $\gamma$  amino acid coding sequence, where said 15 to 172 amino acids are contiguous in native interferon- $\gamma$ . Such 15-172 amino acid regions can also be assembled into polypeptides where two or more such interferon- $\gamma$  regions are joined that are normally discontinuous in the native protein.

*Treating* a disease refers to administering a therapeutic substance effective to reduce the symptoms of the disease and/or lessen the severity of the disease.

## II. Interferon-tau (IFN $\tau$ )

### A. Introduction

The first IFN $\tau$  to be identified was ovine IFN $\tau$  (OvIFN $\tau$ ). Several isoforms of the 18-19 kDa protein were identified in conceptus (the embryo and surrounding membranes) homogenates (Martal, *et al.*, 1979). Subsequently, a low molecular weight protein released into conceptus culture medium was purified and shown to be both heat labile and susceptible to proteases (Godkin, *et al.*, 1982). OvIFN $\tau$  was originally called ovine trophoblast protein-one (oTP-1) because it was the primary secretory protein initially produced by trophectoderm of the sheep conceptus during the critical period of maternal recognition in sheep. One isolate of mature OvIFN $\tau$  is 172 amino acids in length (SEQ ID NO:2).

IFN $\tau$ s with similar characteristics and activities have been isolated from other ruminant species including cows and goats (Bartol, *et al.*, 1985; Gnatek, *et al.*, 1989; Helmer, *et al.*, 1987; and Imakawa, *et al.*, 1989). Bovine IFN $\tau$  (BoIFN $\tau$ ) and OvIFN $\tau$  have (i) have similar functions in maternal recognition of pregnancy, and (ii) share a high degree of amino acid and nucleotide sequence homology between mature proteins. The nucleic acid sequence homology between OvIFN $\tau$  and BoIFN $\tau$  is 76.3% for the 5' non-coding region, 89.7% for the coding region, and 91.9% for the 3' non-coding region. The amino acid sequence homology is 80.4%.

Antisera to all the IFN $\tau$ s cross-react. This is not unexpected since the species specific forms of IFN $\tau$  are more closely homologous to each other than to the IFN $\alpha$ s from the identical species (Roberts, *et al.*, 1992). Relative to other interferons, OvIFN $\tau$  shares about 45 to 68% amino acid homology with Interferon- $\alpha$  and the greatest sequence similarity with the interferon- $\omega$ s (IFN $\omega$ s) of about 68%.

**Table 1**  
**Overview of the Interferons**

Aspects	Type I			Type II
Types	$\alpha$ & $\omega$	$\beta$	$\tau$	$\gamma$
Produced by:	leukocyte	fibroblast	trophoblast	lymphocyte
Effects:				
Antiviral	+	+	+	+
Antiproliferative	+	+	+	+
Pregnancy Signally	-	-	+	-

While IFN $\tau$  displays many of the activities classically associated with type I IFNs (see Table 1, above), considerable differences exist between it and the other type I IFNs. The most

prominent difference is its role in pregnancy, detailed above. Also different is viral induction. All type I IFNs, except IFN $\gamma$ , are induced readily by virus and dsRNA (Roberts, *et al.*, 1992). Induced IFN $\alpha$  and IFN $\beta$  expression is transient, lasting approximately a few hours. In contrast, IFN $\gamma$  synthesis, once induced, is maintained over a period of days (Godkin, *et al.*, 1982). On a  
5 per-cell basis, 300-fold more IFN $\gamma$  is produced than other type I IFNs (Cross and Roberts, 1991).

Other differences may exist in the regulatory regions of the IFN $\gamma$  gene. For example, transfection of the human trophoblast cell line JAR with the gene for bovine IFN $\gamma$  resulted in antiviral activity while transfection with the bovine IFN $\beta$  gene did not. This implies unique transacting factors involved in IFN $\gamma$  gene expression. Consistent with this is the observation that  
10 while the proximal promoter region (from 126 to the transcriptional start site) of IFN $\gamma$  is highly homologous to that of IFN $\alpha$  and IFN $\beta$ ; the region from -126 to -450 is not homologous and enhances only IFN $\gamma$  expression (Cross and Roberts, 1991). Thus, different regulatory factors appear to be involved in IFN $\gamma$  expression as compared with the other type I IFNs.

IFN $\gamma$  expression may also differ between species. For example, although IFN $\gamma$  expression  
15 is restricted to a particular stage (primarily days 13-21) of conceptus development in ruminants (Godkin, *et al.*, 1982), preliminary studies suggest that the human form of IFN $\gamma$  is constitutively expressed throughout pregnancy (Whaley, *et al.*, 1994).

#### B. Production of IFN $\gamma$

IFN $\gamma$  polypeptides suitable for use in the methods of the present invention may produced in  
20 any of a number of ways. For example, they may be purified from animal tissues in which they are expressed, synthesized using a peptide synthesizer or produced recombinantly.

Recombinant IFN $\gamma$  protein may be produced from any selected IFN $\gamma$  polynucleotide fragment using a suitable expression system, such as bacterial or yeast cells. The isolation of IFN $\gamma$  nucleotide and polypeptide sequences is described in Bazer, *et al.* (1994). For example, Bazer,  
25 *et al.*, describe the identification and isolation of a human IFN $\gamma$  gene. A synthetic nucleotide sequence encoding a mature human interferon- $\gamma$  (HuIFN $\gamma$ ) protein is presented herein as SEQ ID NO:3. SEQ ID NO:4 is the corresponding amino acid sequence for a mature HuIFN $\gamma$ 1 protein. SEQ ID NO:5 is the nucleotide sequence, excluding leader sequence, of genomic DNA clone HuIFN $\gamma$ 3, a natural HuIFN $\gamma$  gene, and SEQ ID NO:6 is the predicted amino acid sequence of a  
30 mature human IFN $\gamma$  protein encoded by the sequence represented as SEQ ID NO:5.

To make an IFN $\gamma$  expression vector, an IFN $\gamma$  coding sequence (*e.g.*, SEQ ID NO:1) is placed in an expression vector, *e.g.*, a bacterial expression vector, and expressed according to standard methods. Examples of suitable vectors include lambda gt11 (Promega, Madison WI); pGEX (Smith, *et al.*, 1985); pGEMEX (Promega); and pBS (Stratagene, La Jolla CA) vectors. Other

bacterial expression vectors containing suitable promoters, such as the T7 RNA polymerase promoter or the tac promoter, may also be used. Cloning of the OvIFN $\gamma$  synthetic polynucleotide into a modified pIN III omp-A expression vector is described in the Materials and Methods.

For the experiments described herein, the OvIFN $\gamma$  coding sequence present in SEQ ID NO:1 was cloned into a vector, suitable for transformation of yeast cells, containing the methanol-regulated alcohol oxidase (AOX) promoter and a Pho1 signal sequence. The vector was used to transform *P. pastoris* host cells and transformed cells were used to express the protein according to the manufacturer's instructions.

Other yeast vectors suitable for expressing IFN $\gamma$  for use with methods of the present invention include 2 micron plasmid vectors (Ludwig, *et al.*, 1993), yeast integrating plasmids (YIps; *e.g.*, Shaw, *et al.*, 1988), YEP vectors (Shen, *et al.*, 1986), yeast centromere plasmids (YCps; *e.g.*, Ernst, 1986), and other vectors with regulatable expression (Hitzeman, *et al.*, 1988; Rutter, *et al.*, 1988; Oeda, *et al.*, 1988). Preferably, the vectors include an expression cassette containing an effective yeast promoter, such as the MF $\alpha$ 1 promoter (Ernst, 1986; Bayne, *et al.*, 1988, GADPH promoter (glyceraldehyde -3-phosphate-dehydrogenase; Wu, *et al.*, 1991) or the galactose-inducible GAL10 promoter (Ludwig, *et al.*, 1993; Feher, *et al.*, 1989; Shen, *et al.*, 1986). The yeast transformation host is typically *Saccharomyces cerevisiae*, however, as illustrated above, other yeast suitable for transformation can be used as well (*e.g.*, *Schizosaccharomyces pombe*, *Pichia pastoris* and the like).

Further, a DNA encoding an IFN $\gamma$  polypeptide can be cloned into any number of commercially available vectors to generate expression of the polypeptide in the appropriate host system. These systems include the above described bacterial and yeast expression systems as well as the following: baculovirus expression (Reilly, *et al.*, 1992; Beames, *et al.*, 1991; Clontech, Palo Alto CA); plant cell expression, transgenic plant expression (*e.g.*, Gelvin and Schilperroot, 1988), and expression in mammalian cells (Clontech, Palo Alto CA; Gibco-BRL, Gaithersburg MD). These recombinant polypeptides can be expressed as fusion proteins or as native proteins. A number of features can be engineered into the expression vectors, such as leader sequences which promote the secretion of the expressed sequences into culture medium. The recombinantly produced polypeptides are typically isolated from lysed cells or culture media. Purification can be carried out by methods known in the art including salt fractionation, ion exchange chromatography, and affinity chromatography. Immunoaffinity chromatography can be employed, as described above, using antibodies generated based on the IFN $\gamma$  polypeptides.

In addition to recombinant methods, IFN $\gamma$  proteins or polypeptides can be isolated from selected cells by affinity-based methods, such as by using appropriate antibodies. Further, IFN $\gamma$  peptides may be chemically synthesized using methods known to those skilled in the art.

### III. Effectiveness of Orally-Administered IFN $\gamma$

5 Experiments performed in support of the present invention and detailed below demonstrate that orally-administered IFN $\gamma$  polypeptide compositions are comparable in efficacy to injected IFN $\gamma$  compositions with respect to the treatment of diseases or disease conditions which benefit from treatment with IFN $\gamma$ .

Not only was orally-administered IFN $\gamma$  effective at treating a disease benefiting from IFN $\gamma$  10 treatment (EAE), but the oral route of administration resulted in unexpected advantages relative to treatment with injected IFN $\gamma$  compositions. For example, orally-administered IFN $\gamma$  resulted in a significantly lower level of anti-IFN $\gamma$  antibodies in the serum of treated individuals (see Example 7). This is beneficial because the orally-administered IFN $\gamma$  is therefore less likely to be rendered ineffective by a host immune response (*i.e.*, desensitization to the treatment and/or dose level is 15 significantly decreased), and the individual receiving the treatment is less likely to suffer adverse side effects as a result of such an immune response.

Results of experiments demonstrating these and related findings are presented below.

#### A. Orally-Administered IFN $\gamma$ Inhibits Development of EAE

The efficacy of IFN $\gamma$  in treating autoimmune disorders may be evaluated in rodents with 20 experimental allergic encephalomyelitis (EAE; Zamvil and Steinman, 1990), an animal model of antigen-induced autoimmunity. EAE resembles human multiple sclerosis (MS) both in its clinical and pathological manifestations and can thus be used to assess treatments for human autoimmune diseases such as MS. EAE is a T-cell-mediated inflammatory autoimmune demyelinating disease induced by immunizing susceptible mouse, rat or guinea pig strains with myelin basic protein 25 (MBP) or with encephalitogenic peptide fragments. Genetic susceptibility in the model animal strains is based in part on the capacity of encephalitogenic peptides to bind to particular class II major histocompatibility complex (MHC-II) molecules (Fritz, *et al.*, 1983; Wraith, *et al.*, 1989). In particular, mice having the H-2<sup>d</sup> haplotype are susceptible to EAE. Susceptible mouse strains include PL/J mice (Klein, *et al.*, 1983), (PL/J  $\times$  SJL)F<sub>1</sub> mice (Zamvil and Steinman, 1990; 30 Wraith, *et al.*, 1989), B10.PL mice (Figuro, *et al.*, 1982), NZW mice (Kotzin, *et al.*, 1987), and (NZB  $\times$  NZW)F<sub>1</sub> (Kotzin, *et al.*, 1987) mice.

Gamma-interferon (IFN $\gamma$ ) and beta-interferon (IFN $\beta$ ) have been demonstrated to be effective in treating multiple sclerosis (Johnson, *et al.*, 1994; IFN $\beta$  Multiple Sclerosis Study Group, 1993).

In fact, IFN $\beta$  has been approved by the FDA as a therapeutic for multiple sclerosis. Although  $\beta$ -IFN is effective against MS, it has relatively high toxicity, and as a result, has a variety of undesirable side effects. As described herein, however, IFN $\gamma$  has significantly lower toxicity than other interferons and may therefore exhibit fewer undesirable side effects.

5 In experiments performed in support of the present invention and detailed in Example 1, orally-administered and injected IFN- $\gamma$  was tested for its ability to prevent the induction of EAE. EAE was induced in New Zealand White (NZW) mice by immunization with bovine myelin basic protein (bMBP). Recipient NZW mice received OvIFN $\gamma$  by either i.p. injection or oral feeding 48 hours prior to, on the day of, and 48 hours after immunization with bovine myelin basic protein  
10 (bMBP) for induction of experimental allergic encephalomyelitis (EAE).

Both oral feeding and i.p. injection of OvIFN $\gamma$  protected against EAE (Example 1, Table 3). All animals that received IFN $\gamma$  via i.p. injection, and 7 of 9 animals that received IFN $\gamma$  orally, were protected from symptoms of EAE. Furthermore, anti-OvIFN $\gamma$  monoclonal antibody HL127 was effective at partially neutralizing the ability of the OvIFN $\gamma$  to block EAE. These experiments  
15 demonstrate that orally-administered IFN $\gamma$  is effective in treating symptoms of EAE, an animal model of multiple sclerosis.

#### B. OvIFN $\gamma$ is Present in Sera Following Oral Administration.

To confirm that orally-administered IFN $\gamma$  enters the circulation, the sera of mice that received IFN $\gamma$  by i.p. injection or by oral administration were tested for the presence of IFN $\gamma$  using a  
20 cytopathic effect (antiviral) assay (Familetti, *et al.*, 1981) as described in Example 2.

The results are shown in Fig. 1. Specific activities are expressed in antiviral units/mg protein obtained from antiviral assays using MDBK cells. OvIFN $\gamma$  was detected for up to two 2 hours following oral feeding (filled bars) at levels of 200 U/ml. These data indicate that orally-administered IFN $\gamma$  enters the circulation and remains in serum for about two hours after being  
25 administered.

#### C. Lack of Toxicity from Orally-administered OvIFN $\gamma$

It has been previously demonstrated that the type I IFNs IFN $\alpha$  and IFN $\beta$  induced toxic side effects manifested as flu like symptoms, fever, nausea and malaise when used as therapeutics in humans (Degre, 1974; Fent and Zbinden, 1987). In contrast, OvIFN $\gamma$  exhibits a remarkable lack  
30 of toxicity both *in vitro* and *in vivo*. Experiments performed in support of the present invention compared OvIFN $\gamma$  with IFNs  $\alpha$  and  $\beta$  for induction of toxicity as measured by lymphocyte depression in peripheral blood when given via oral feeding. Blood was obtained from the tail and white blood cells (WBC) counts were enumerated using a hemocytometer. Differential WBC counts were performed on Wright-Giemsa-stained blood smears.

The results are shown in Tables 2a, 2b and 2c, below. Significant levels of toxicity were detected in mice fed either IFN  $\alpha$  and  $\beta$  while no significant lymphocyte depression was detected in mice fed  $10^5$ ,  $2 \times 10^5$  or  $5 \times 10^5$  U of OvIFN $\tau$  or PBS alone. These data suggest that orally-administered OvIFN $\tau$  has significantly-reduced toxicity with respect to other type I IFNs.

5

**Tables 2a-2c**  
**Comparison of IFNs  $\tau$ ,  $\beta$  and  $\alpha$  for Toxicity After Oral Feeding**

**Table 2a**

IFN (DOSE)	CELL COUNT (CELL NO. $\times 10^3$ )	
	BEFORE ORAL FEEDING	
	TOTAL WBC	LYMPHOCYTES
PBS	$7.0 \pm 1.4$	$6.1 \pm 1.2$
$\tau(10^5)$	$7.5 \pm 0.7$	$6.4 \pm 0.6$
$\tau(2 \times 10^5)$	$6.5 \pm 0.7$	$5.3 \pm 0.6$
$\tau(5 \times 10^5)$	$7.5 \pm 0.7$	$6.5 \pm 0.6$
$\beta(10^5)$	$7.0 \pm 0.7$	$5.9 \pm 1.2$
$\beta(2 \times 10^5)$	$7.5 \pm 2.1$	$6.5 \pm 1.8$
$\alpha(10^5)$	$7.5 \pm 0.7$	$6.6 \pm 0.6$

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**Table 2b**

IFN (DOSE)	CELL COUNT (CELL NO. $\times 10^3$ )		
	18 H AFTER ORAL FEEDING		
	TOTAL WBC	LYMPHOCYTES	% LYMPHOCYTE DEPRESSION
PBS	—	—	—
$\tau(10^5)$	$7.0 \pm 1.4$	$6.0 \pm 1.3$	6.2
$\tau(2 \times 10^5)$	$7.0 \pm 2.8$	$5.9 \pm 2.4$	0
$\tau(5 \times 10^5)$	$7.5 \pm 2.1$	$6.3 \pm 1.8$	3.1
$\beta(10^5)$	$6.5 \pm 0.7$	$5.1 \pm 0.6$	13.6
$\beta(2 \times 10^5)$	$6.5 \pm 0.7$	$4.1 \pm 0.4^\dagger$	37.0
$\alpha(10^5)$	$6.5 \pm 2.1$	$4.7 \pm 1.6$	28.8

20

25

 $^\dagger p < 0.05$



Table 2c

IFN (DOSE)	CELL COUNT (CELL NO. $\times 10^3$ )		
	24 H AFTER ORAL FEEDING		
	TOTAL WBC	LYMPHOCYTES	% LYMPHOCYTE DEPRESSION
PBS	$7.5 \pm 0.7$	$6.4 \pm 0.6$	0
$\tau(10^5)$	$8.0 \pm 2.8$	$6.9 \pm 2.4$	0
$\tau(2 \times 10^5)$	$7.0 \pm 1.4$	$6.0 \pm 1.1$	0
$\tau(5 \times 10^5)$	$8.0 \pm 4.2$	$7.0 \pm 3.6$	0
$\beta(10^5)$	$6.5 \pm 3.5$	$5.1 \pm 2.8$	13.6
$\beta(2 \times 10^5)$	$6.5 \pm 0.7$	$4.0 \pm 0.4^{\dagger}$	38.5
$\alpha(10^5)$	$7.0 \pm 0$	$5.0 \pm 0^{\ddagger}$	24.2

<sup>†</sup>p < 0.05<sup>‡</sup>p < 0.03

#### D. OvIFN $\tau$ Prevents Chronic Relapse of EAE

In addition to preventing the onset of symptoms associated with EAE, orally-administered OvIFN $\tau$  prevents paralysis in a chronic-relapsing model of EAE, as detailed in Example 3.

- Whereas 5/5 mice immunized with MBP (to induce EAE) which did not receive OvIFN $\tau$  treatment developed chronic relapsing paralysis, 4/5 animals treated with OvIFN $\tau$  (either i.p. injection or oral feeding, administered every 48 hours) were fully protected from the disease (Figs. 2B and 2C). These data further support the results described above, and indicate that oral administration of IFN $\tau$  can block the development of chronic relapsing EAE. The experiments also suggest that orally-administration of IFN $\tau$  as infrequently as once every 48 hours, over an extended period of time, is as effective as i.p. injection at treating a disease condition responsive to treatment by interferon-tau.

#### E. Histological Analyses of Spinal Chord from EAE Mice following Oral Administration of IFN $\tau$ .

- The ability of OvIFN $\tau$  to prevent EAE was also assayed by analyzing the effect of OvIFN $\tau$  treatment on cellular consequences of the disease, manifested in the central nervous system (CNS) as lymphocytic lesions in spinal cord white matter. The lesions are indicative of the extent of lymphocyte infiltration into the CNS. MBP-immunized mice were either not treated (control) or treated with OvIFN $\tau$  by oral or i.p. routes, and sections of the spinal cord lumbar region were stained and evaluated for lymphocytes as described in Example 4. Lymphocytic lesions were

present in spinal cord white matter of control animals (Fig. 3A), but not in mice treated with OvIFN $\gamma$  by i.p. injection (Fig. 3B) or oral feeding (Fig. 3C). These data indicate that the protective effect of IFN $\gamma$  is associated with inhibition of lymphocyte infiltration of the CNS. Further, the data demonstrate that IFN $\gamma$  treatment inhibits cellular manifestation of the autoimmune disease, rather than simply masking symptoms.

F. Cessation of Treatment with OvIFN $\gamma$  Results in Relapsing Paralysis.

Experiments detailed in Example 6 were performed to determine the type and duration of treatment effective to prevent EAE in mice injected with MBP. The mice were protected from EAE by OvIFN $\gamma$  treatment via i.p. injection or oral feeding (every 48 hours) as long as the treatment persisted (58 days in Example 6), but developed symptoms of the disease after OvIFN $\gamma$  treatment was stopped (Figure 5). These results suggest that while IFN $\gamma$  may not cure an autoimmune condition like EAE (*e.g.*, MS), it is an effective treatment that inhibits the pathological manifestations of the condition so long as treatment is continued.

G. Oral Administration of OvIFN $\gamma$  Reduces Anti-OvIFN $\gamma$  Antibody Response.

As detailed in Example 7, one advantage of orally-administered (as opposed to injected) IFN $\gamma$  treatment is a reduction in the anti-IFN $\gamma$  antibody titer in individuals receiving the oral treatment. After removal of OvIFN $\gamma$  treatment, mice from each treatment group were bled and sera were examined for the presence of anti-OvIFN $\gamma$  antibodies by ELISA. Whereas mice receiving IFN $\gamma$  by i.p. injection exhibited elevated levels of anti-IFN $\gamma$  antibodies, animals receiving IFN $\gamma$  by oral feeding exhibited much lower anti-IFN $\gamma$  antibody titers (typically 3 to 5 -fold lower). As expected mice which received no OvIFN $\gamma$  treatment displayed no anti-OvIFN $\gamma$  antibodies.

The sera were also examined for their ability to neutralize OvIFN $\gamma$  antiviral activity on the MDBK cell line. None of the sera from either i.p. injected or orally fed mice possessed neutralizing activity (Table 4). These results suggest that oral feeding of OvIFN $\gamma$  largely circumvents an antibody response directed against the OvIFN $\gamma$  protein. Such a reduced antibody response in orally-treated subjects reduces the chance of undesirable immune system-related side effects of IFN $\gamma$  treatment.

IV. Applications

A. IFN $\gamma$  as a Treatment for Immune System Disorders

Diseases which may be treated using methods of the present invention include autoimmune, inflammatory, proliferative and hyperproliferative diseases, as well as cutaneous manifestations of immunologically mediated diseases. In particular, methods of the present invention are advantageous for treating conditions relating to immune system hypersensitivity. There are four

types of immune system hypersensitivity (Clayman, 1991). Type I, or immediate/anaphylactic hypersensitivity, is due to mast cell degranulation in response to an allergen (*e.g.*, pollen), and includes asthma, allergic rhinitis (hay fever), urticaria (hives), anaphylactic shock, and other illnesses of an allergic nature. Type II, or autoimmune hypersensitivity, is due to antibodies that are directed against perceived "antigens" on the body's own cells. Type III hypersensitivity is due to the formation of antigen/antibody immune complexes which lodge in various tissues and activate further immune responses, and is responsible for conditions such as serum sickness, allergic alveolitis, and the large swellings that sometimes form after booster vaccinations. Type IV hypersensitivity is due to the release of lymphokines from sensitized T-cells, which results in an inflammatory reaction. Examples include contact dermatitis, the rash of measles, and "allergic" reactions to certain drugs.

The mechanisms by which certain conditions may result in hypersensitivity in some individuals are generally not well understood, but may involve both genetic and extrinsic factors. For example, bacteria, viruses or drugs may play a role in triggering an autoimmune response in an individual who already has a genetic predisposition to the autoimmune disorder. It has been suggested that the incidence of some types of hypersensitivity may be correlated with others. For example, it has been proposed that individuals with certain common allergies are more susceptible to autoimmune disorders.

Autoimmune disorders may be loosely grouped into those primarily restricted to specific organs or tissues and those that affect the entire body. Examples of organ-specific disorders (with the organ affected) include multiple sclerosis (myelin coating on nerve processes), type I diabetes mellitus (pancreas), Hashimoto's thyroiditis (thyroid gland), pernicious anemia (stomach), Addison's disease (adrenal glands), myasthenia gravis (acetylcholine receptors at neuromuscular junction), rheumatoid arthritis (joint lining), uveitis (eye), psoriasis (skin), Guillain-Barré Syndrome (nerve cells) and Grave's disease (thyroid). Systemic autoimmune diseases include systemic lupus erythematosus and dermatomyositis.

Other examples of hypersensitivity disorders include asthma, eczema, atopic dermatitis, contact dermatitis, other eczematous dermatitides, seborrheic dermatitis, rhinitis, Lichen planus, Pemphigus, bullous Pemphigoid, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Alopecia areata, atherosclerosis, primary biliary cirrhosis and nephrotic syndrome. Related diseases include intestinal inflammations, such as Coeliac disease, proctitis, eosinophilia gastroenteritis, mastocytosis, inflammatory bowel disease, Crohn's disease and ulcerative colitis, as well as food-related allergies.

Autoimmune diseases particularly amenable for treatment using the methods of the present invention include multiple sclerosis, type I (insulin dependent) diabetes mellitus, lupus erythematosus, amyotrophic lateral sclerosis, Crohn's disease, rheumatoid arthritis, stomatitis, asthma, uveitis, allergies and psoriasis.

- 5       Methods of the present invention may be used to therapeutically treat and thereby alleviate autoimmune disorders such as those discussed above. These treatments are exemplified herein with respect to the treatment of EAE, an animal model for multiple sclerosis.

B.    IFN $\gamma$  as Treatment for Reproductive Disorders.

- 10       Although IFN $\gamma$  bears some similarity to the IFN $\alpha$  family based on structure and its potent antiviral properties, the IFN $\alpha$ s do not possess the reproductive properties associated with IFN $\gamma$ . For example, recombinant human IFN $\alpha$  had no effect on interestrus interval compared to IFN $\gamma$ , even when administered at twice the dose (Davis, *et al.*, 1992).

- 15       Therefore, although IFN $\gamma$  has some structural similarities to other interferons, it has very distinctive properties of its own: for example, the capability of significantly influencing the biochemical events of the estrous cycle.

- 20       The IFN $\gamma$  compositions of the present invention can be used in methods of enhancing fertility and prolonging the life span of the *corpus luteum* in female mammals as generally described in Hansen, *et al.* (1991). According to the teachings herein, such methods of enhancing fertility include oral administration of IFN $\gamma$  in a therapeutically-effective amount. Further, the compositions may be similarly employed to regulate growth and development of uterine and/or fetal-placental tissues. Compositions containing human IFN $\gamma$  are particularly useful for treatment of humans, since potential antigenic responses are less likely using a same-species protein.

C.    IFN $\gamma$  as an Antiviral Treatment

- 25       The antiviral activity of IFN $\gamma$  has broad therapeutic applications without the toxic effects that are usually associated with IFN $\alpha$ s. As described above, IFN $\gamma$  exerts its therapeutic activity without adverse effects on the cells. The relative lack of cytotoxicity of IFN $\gamma$  makes it extremely valuable as an *in vivo* therapeutic agent and sets IFN $\gamma$  apart from most other known antiviral agents and all other known interferons.

- 30       Formulations containing IFN $\gamma$  can be orally-administered to inhibit viral replication. Further, the compositions can be employed in methods for affecting the immune relationship between fetus and mother, for example, in preventing transmission of maternal viruses (*e.g.*, HIV) to the developing fetus. Compositions containing a human interferon- $\gamma$  are particularly useful for

treatment of humans, since potential antigenic responses are less likely using a homologous protein.

Examples of specific viral diseases which may be treated by orally-administered IFN $\gamma$  include, but are not limited to, hepatitis A, hepatitis B, hepatitis C, non-A, non-B, non-C hepatitis, 5 Epstein-Barr viral infection, HIV infection, herpes virus (EB, CML, herpes simplex), papilloma, poxvirus, picorna virus, adeno virus, rhino virus, HTLV I, HTLV II, and human rotavirus.

#### D. IFN $\gamma$ as an Antiproliferative Treatment

IFN $\gamma$  exhibits potent anticellular proliferation activity. Accordingly, pharmaceutical compositions containing IFN $\gamma$ , suitable for oral administration, can be used to inhibit cellular 10 growth without the negative side effects associated with other interferons which are currently known. Such compositions or formulations can be used to inhibit, prevent, or slow tumor growth.

Examples of specific cell proliferation disorders which may be treated by orally-administered IFN $\gamma$  include, but are not limited to, hairy cell leukemia, Kaposi's Sarcoma, chronic myelogenous leukemia, multiple myeloma, superficial bladder cancer, skin cancer (basal cell carcinoma and 15 malignant melanoma), renal cell carcinoma, ovarian cancer, low grade lymphocytic and cutaneous T cell lymphoma, and glioma.

Furthermore, the development of certain tumors is mediated by estrogen. Experiments performed in support of the present invention indicate that IFN $\gamma$  can suppress estrogen receptor numbers. Therefore, the IFN $\gamma$ -containing compositions may be particularly useful in the treatment 20 or prevention of estrogen-dependent tumors.

#### E. Veterinary Applications

In addition to the uses of the methods of the present invention detailed above, it will be appreciated that the methods may be applied to the treatment of a variety of immune system disorders suffered by domesticated and wild animals. For example, hypothyroidism in dogs 25 typically results from a progressive destruction of the thyroid, which may be associated with Lymphocytic thyroiditis (Kemppainen and Clark, 1994). Lymphocytic thyroiditis, which resembles Hashimoto's thyroiditis in humans, is thought to be an autoimmune disorder. According to the guidance presented herein, hypothyroidism due to Lymphocytic thyroiditis in dogs may be treated with IFN $\gamma$  as described above.

30 Another type of autoimmune disorder in dogs that may be alleviated by treatment with IFN $\gamma$  is characterized by antinuclear antibody (ANA) positivity, pyrexia and seronegative arthritis (Day, *et al.*, 1985). Immune-mediated thrombocytopenia (ITP; Kristensen, *et al.*, 1994; Werner, *et al.*, 1985), systemic lupus erythematosus (Kristensen, *et al.*, 1994), and leukopenia and Coomb's

positive hemolytic anemia (Werner, *et al.*, 1985), may also be amenable to treatment using methods of the present invention.

## V. IFN $\gamma$ Pharmaceutical Composition Useful for Oral Administration

### A. Formulation

5 Therapeutic preparations containing IFN $\gamma$  or related polypeptides or proteins can be formulated according to known methods for preparing pharmaceutically useful compositions. Formulations comprising polypeptides like interferons have been previously described (*e.g.*, Martin, 1976). In general, the IFN $\gamma$  therapeutic compositions are formulated such that an effective amount of the IFN $\gamma$  is combined with a suitable additive, carrier and/or excipient in order to  
10 facilitate effective oral administration of the composition. For example, tablets and capsules containing IFN $\gamma$  may be prepared by combining IFN $\gamma$  (*e.g.*, lyophilized IFN $\gamma$  protein) with additives such as pharmaceutically acceptable carriers (*e.g.*, lactose, corn starch, light silicic anhydride, microcrystalline cellulose, sucrose), binders (*e.g.*, alpha-form starch, methylcellulose, carboxymethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, polyvinylpyr-  
15 rolidone), disintegrating agents (*e.g.*, carboxymethylcellulose calcium, starch, low substituted hydroxy-propylcellulose), surfactants (*e.g.*, Tween 80, polyoxyethylene-polyoxypropylene copolymer), antioxidants (*e.g.*, L-cysteine, sodium sulfite, sodium ascorbate), lubricants (*e.g.*, magnesium stearate, talc), or the like.

Further, IFN $\gamma$  polypeptides of the present invention can be mixed with a solid, pulverulent  
20 or other carrier, for example lactose, saccharose, sorbitol, mannitol, starch, such as potato starch, corn starch, millopectine, cellulose derivative or gelatine, and may also include lubricants, such as magnesium or calcium stearate, or polyethylene glycol waxes compressed to the formation of tablets. By using several layers of the carrier or diluent, tablets operating with slow release can be prepared.

25 Liquid preparations for oral administration can be made in the form of elixirs, syrups or suspensions, for example solutions containing from about 0.1% to about 30% by weight of IFN $\gamma$ , sugar and a mixture of ethanol, water, glycerol, propylene, glycol and possibly other additives of a conventional nature.

### B. Dosage

30 An orally-active IFN $\gamma$  pharmaceutical composition is administered in a therapeutically effective amount to an individual in need of treatment. The dose may vary considerably and is dependent on factors such as the seriousness of the disorder, the age and the weight of the patient, other medications that the patient may be taking and the like. This amount or dosage is typically

determined by the attending physician. The dosage will typically be between about  $1 \times 10^5$  and  $1 \times 10^8$  units/day, preferably between about  $1 \times 10^6$  and  $1 \times 10^7$  units/day. It will be appreciated that because of its lower toxicity, IFN $\gamma$  can be administered at higher doses than, for example, IFN $\beta$ . By way of comparison, patients with multiple sclerosis (MS) were treated with  $10^6$  U and  $8 \times 10^6$  U of IFN $\beta$ . Patients receiving  $8 \times 10^6$  U suffered fewer relapses of disease than did patients receiving  $10^6$  U. However, patients receiving the higher dose of IFN $\beta$  ( $8 \times 10^6$  U) also exhibited more side-effects associated with IFN $\beta$ 's toxicity. In view of the lower toxicity of IFN $\gamma$ , these higher effective dosages could be administered without the associated toxic side-effects.

Disorders requiring a steady elevated level of IFN $\gamma$  in plasma will benefit from administration as often as about every two to four hours, while other disorders, such as MS, may be effectively treated by administering a therapeutically-effective dose at less frequent intervals, *e.g.*, once every 48 hours. The rate of administration of individual doses is typically adjusted by an attending physician to enable administration of the lowest total dosage while alleviating the severity of the disease being treated.

Once improvement of a patient's condition has occurred, a maintenance dose is administered if necessary. Subsequently, the dosage or the frequency of administration, or both, may be reduced, as a function of the symptoms, to a level at which the improved condition is retained.

#### C. Combination Therapies

It will, of course, be understood that the compositions and methods of this invention may be used in combination with other therapies. For example, in view of IFN $\gamma$ 's relative lack of toxicity at high dosages, MS patients that do not show improvement at IFN $\beta$ 1b's low dosage or could not tolerate IFN $\beta$ 1b due to toxicity may benefit from subsequent or simultaneous treatment with higher dosages of IFN $\gamma$  or peptides derived therefrom. Further, development of neutralizing antibodies has been demonstrated in IFN $\beta$ 1b treated patients (Weinstock-Guttman, *et al.*, 1995). In cases where such neutralizing antibodies prove to impede the effectiveness of IFN $\beta$ 1b, IFN $\gamma$  may be an important alternative therapy, since antibody cross-reactivity is unlikely to occur, and IFN $\gamma$  is unlikely to generate neutralizing antibodies (see Example 7). Orally-administered IFN $\gamma$  is particularly advantageous in this respect, since it causes a significantly lower anti-IFN $\gamma$  antibody response than injected IFN $\gamma$ .

Another type of combination therapy enabled by the present invention is the oral administration of an antigen against which an autoimmune response is directed in combination with IFN $\gamma$ . Oral administration of such an antigen can result in tolerization, reducing the severity of the autoimmune disease (for review, see, *e.g.*, Weiner, *et al.*, 1994). It is contemplated that the

IFN $\gamma$  has a synergistic effect with the tolerization induced by the antigen, thereby alleviating the severity of the autoimmune-disease. For example, MBP has been shown to suppress EAE (Lider, *et al.*, 1989). According to the methods of the present invention, MBP may be administered in combination with IFN $\gamma$  to treat multiple sclerosis. Other examples include administration of IFN $\gamma$  with collagen to treat rheumatoid arthritis, and with acetylcholine receptor polypeptides to treat myasthenia gravis.

Furthermore, IFN $\gamma$  may be orally administered with known immunosuppressants, such as steroids, to treat autoimmune diseases such a multiple sclerosis. The immunosuppressants may act synergistically with IFN $\gamma$  and result in a more effective treatment that could be obtained with an equivalent dose of IFN $\gamma$  or the immunosuppressant alone.

Similarly, in a treatment for a cancer or viral disease, IFN $\gamma$  may be administered in conjunction with, *e.g.*, a therapeutically effective amount of one or more chemotherapy agents such as busulfan, 5-fluoro-uracil (5-FU), zidovudine (AZT), leucovorin, melphalan, prednisone, cyclophosphamide, dacarbazine, cisplatin, and dipyridamole.

The following examples illustrate but in no way are intended to limit the present invention.

## VI. Materials and Methods

### A. Buffers

#### Phosphate-buffered saline (PBS)

10  $\times$  stock solution, 1 liter:

80 g NaCl

2 g KCl

11.5 g Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O

2 g KH<sub>2</sub>PO<sub>4</sub>

Working solution, pH 7.3:

137 mM NaCl

2.7 mM KCl

4.3 mM Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O

1.4 mM KH<sub>2</sub>PO<sub>4</sub>

### B. General ELISA Protocol for Detection of Antibodies

Polystyrene 96 well plates Immulon II (PGC) were coated with 5  $\mu$ g/mL (100  $\mu$ L per well) antigen in 0.1 M carbonate/bicarbonate buffer, pH 9.5. The plates were sealed with parafilm and stored at 4°C overnight.

Following incubation, the plates were aspirated and blocked with 300  $\mu$ L 10% NGS and incubated at 37°C for 1 hr. The plates were then washed 5 times with PBS 0.5% "TWEEN-20". Antisera were diluted in 0.1 M PBS, pH 7.2. The desired dilution(s) of antisera (0.1 mL) were



added to each well and the plates incubated 1 hour at 37°C. The plates were then washed 5 times with PBS 0.5% "TWEEN-20".

Horseradish peroxidase (HRP) conjugated goat anti-human antiserum (Cappel, Durham, NC) was diluted 1/5,000 in PBS. 0.1 mL of this solution was added to each well. The plate was  
5 incubated 30 min at 37°C, then washed 5 times with PBS.

Sigma ABTS (substrate) was prepared just prior to addition to the plate. The reagent consists of 50 mL 0.05 M citric acid, pH 4.2, 0.078 mL 30% hydrogen peroxide solution and 15 mg ABTS. 0.1 mL of the substrate was added to each well, then incubated for 30 min at room temperature. The reaction was stopped with the addition of 0.050 mL 5% SDS (w/v). The  
10 relative absorbance is determined at 410 nm.

### C. Production of OvIFN- $\gamma$

A synthetic OvIFN $\gamma$  gene was generated using standard molecular methods (Ausubel, *et al.*, 1988) by ligating oligonucleotides containing contiguous portions of a DNA sequence encoding the OvIFN $\gamma$  amino acid sequence (Imakawa, *et al.*, 1987). The resulting IFN $\gamma$  polynucleotide  
15 coding sequence spans position 16 through 531: a coding sequence of 172 amino acids.

The full length synthetic gene *Sma*I/*Sst*I fragment (540 bp) was cloned into a modified pIN III omp-A expression vector and transformed into a competent SB221 strain of *E. coli*. For expression of the IFN $\gamma$  protein, cells carrying the expression vector were grown in L-broth containing ampicillin to an OD (550 nm) of 0.1-1, induced with IPTG (isopropyl-1-thio- $\beta$ -D-galactoside) for 3 hours and harvested by centrifugation. Soluble recombinant IFN $\gamma$  was liberated  
20 from the cells by sonication or osmotic fractionation.

For expression in yeast, the IFN $\gamma$  gene was amplified using polymerase chain reaction (PCR; Mullis, 1987; Mullis, *et al.*, 1987) with PCR primers containing *Sma*I and *Sac*I restriction sites at the 5' and 3' ends, respectively. The amplified fragments were digested with *Sma*I and *Sac*II and  
25 ligated into the *Sac*II and *Sma*I sites of "pBLUESCRIPT+(KS)", generating pBSY-IFN $\gamma$ .

Plasmid pBSY-IFN $\gamma$  was digested with *Sac*II and *Eco*RV and the fragment containing the synthetic IFN $\gamma$  gene was isolated. The yeast expression vector pBS24Ub (Sabin, *et al.*, 1989; Ecker, *et al.*, 1989) was digested with *Sac*II. Blunt ends were generated using T4 DNA polymerase. The vector DNA was extracted with phenol and ethanol precipitated (Sambrook, *et al.*, 1989). The recovered plasmid was digested with *Sac*II, purified by agarose gel electro-  
30 phoresis, and ligated to the *Sac*II-*Eco*RV fragment isolated from pBSY-IFN $\gamma$ . The resulting recombinant plasmid was designated pBS24Ub-IFN $\gamma$ .

The recombinant plasmid pBS24Ub-IFN $\gamma$  was transformed into *E. coli*. Recombinant clones containing the IFN $\gamma$  insert were isolated and identified by restriction enzyme analysis. IFN $\gamma$

coding sequences were isolated from pBS24Ub-IFN $\gamma$  and cloned into a *Pichia pastoris* expression vector containing the alcohol oxidase (AOX1) promoter (Invitrogen, San Diego, CA). The vector was then used to transform *Pichia pastoris* GS115 His<sup>-</sup> host cells and protein was expressed following the manufacturer's instructions. The protein was secreted into the medium and purified by successive DEAE-cellulose and hydroxyapatite chromatography to electrophoretic homogeneity as determined by SDS-PAGE and silver staining. The purified protein had a specific activity of about 0.29 to about  $0.44 \times 10^8$  U/mg as measured by anti-viral activity on Madin-Darby bovine kidney (MDBK) cells.

### Example 1

10

#### Orally-Administered OvIFN $\gamma$ Blocks Development of Experimental Allergic Encephalomyelitis

Orally-administered and injected IFN- $\gamma$  was tested for its ability to prevent the induction of EAE. Recipient New Zealand White (NZW) mice received OvIFN $\gamma$  ( $10^5$  U/ml) by either i.p. injection or oral feeding 48 hours prior to, on the day of, and 48 hours after immunization with bovine myelin basic protein (bMBP) for induction of experimental allergic encephalomyelitis (EAE).  $10^5$  U of IFN $\gamma$  were mixed with PBS to a total volume of 100  $\mu$ l and administered using a feeding tube placed down the esophagus and into the stomach. The dilution of the IFN $\gamma$  in PBS was done immediately before administration.

For induction of EAE in NZW mice, 300  $\mu$ g of bovine myelin basic protein (bMBP) was emulsified in complete Freund's adjuvant (CFA) containing 8 mg/ml of H37Ra (*Mycobacterium tuberculosis*, Difco, Detroit, MI) and injected on either side of the base of the tail. On the day of immunization and 48 hours later, 400 ng of Pertussis toxin (List Biologicals, Campbell, CA) was also injected. For induction of EAE in SJL/J mice, the same protocol was used as described except mice were immunized again 7 days after the initial immunization. Mice were examined daily for signs of EAE and severity of disease was graded on the following scale: 1, loss of tail tone; 2, hind limb weakness; 3, paraparesis; 4, paraplegia; 5, moribund/death.

To determine whether prevention of EAE was specific to OvIFN $\gamma$  treatment, an anti-OvIFN $\gamma$  monoclonal antibody (mAb), HL127, was used to neutralize OvIFN $\gamma$  ability to block EAE (antibody HL127, directed against aa 139-172 of SEQ ID NO:2, neutralizes the antiviral activity of OvIFN $\gamma$  in an antiviral assay using the MDBK cell line). A 1:10 dilution of HL127 was incubated for 2 hours with OvIFN $\gamma$  prior to administration by either i.p. injection or oral feeding. Antibodies directed against IFN $\gamma$  antigens, may be generated using the information herein combined with known techniques for antibody production (*e.g.*, Harlow, *et al.*, 1988).

The results are shown in Table 3, below. Both oral feeding and i.p. injection of OvIFN $\gamma$  protected against acute induction of EAE. None of the animals that received IFN $\gamma$  via i.p. injection developed symptoms of EAE, while of the animals that received IFN $\gamma$  orally, 7 of 9 (78%) were protected. Anti-OvIFN $\gamma$  antibody HL127 was effective at partially neutralizing the ability of the OvIFN $\gamma$  to block EAE. These data indicate that orally-administered IFN $\gamma$  is effective as a treatment in an animal model of multiple sclerosis.

**Table 3**  
Oral Feeding of OvIFN $\gamma$  Blocks Acute EAE and Can Be Reversed by an OvIFN $\gamma$  Specific Monoclonal Antibody in NZW Mice

ROUTE OF ADMINISTRATION	TREATMENT	DISEASE INCIDENCE	MEAN DAY OF ONSET	MEAN SEVERITY
i.p.	PBS	4/4	24.8 $\pm$ 2.1	2.5 $\pm$ 0
i.p.	OvIFN $\gamma$	0/4	--	--
i.p.	OvIFN $\gamma$ + HL127	3/4	20.7 $\pm$ 1.2	2.3 $\pm$ 0.6
oral	PBS	7/9	22.0 $\pm$ 1.0	2.7 $\pm$ 0.6
oral	OvIFN $\gamma$	2/9	19	3
oral	OvIFN $\gamma$ + HL127	5/8	20.7 $\pm$ 0.6	3 $\pm$ 0

OvIFN $\gamma$  ( $10^5$  U) was administered 48 hours prior to MBP immunization, on the day of MBP immunization and 48 hours after MBP immunization by either i.p. injection or oral feeding. HL127, a monoclonal antibody specific for OvIFN $\gamma$ , was incubated with OvIFN $\gamma$  for two hours prior to administration.

### Example 2

#### Detection of OvIFN $\gamma$ in Sera Following Oral Administration

The amount of OvIFN $\gamma$  detectable in the sera of mice (treated as above) was compared over time after oral feeding or i.p. injection of OvIFN $\gamma$ . Mice were administered  $3 \times 10^5$  U of OvIFN $\gamma$  and bled at 0.5, 2, 4, 6, 24 and 48 hours following IFN $\gamma$  administration. Sera were tested in a cytopathic effect (viral plaque) assay (Familetti, *et al.*, 1981) to determine the amount of IFN $\gamma$  in the samples.

Briefly, dilutions of IFN $\gamma$  were added to MDBK cells grown to confluency in a flat bottom 96 well plate and incubated for 18 to 24 hours at 37°C. Vesicular stomatitis virus (VSV) was added to the plate for 45 minutes at room temperature. Virus was removed and methyl cellulose was added and the plate incubated for 48 hours at 37°C. After removal of methyl cellulose, the plate was stained with crystal violet for visualization of plaques. For measurement of IFN neutralization, OvIFN $\gamma$  at a concentration of 500 U/ml was incubated for 1 hour at 37°C with either sera or HL127 (a monoclonal specific of OvIFN $\gamma$ ). One antiviral unit caused a 50%

reduction in destruction of the monolayer, relative to untreated MDBK cells infected with VSV (control plates). All samples were assayed simultaneously to eliminate interassay variability.

As shown in Fig. 1, OvIFN $\gamma$  was detected at 0.5 hour and 2 hours after oral feeding (filled bars) at levels of 200 U/ml. By comparison, somewhat higher levels of OvIFN $\gamma$  were detected for over a 24 hour period of time after i.p. injection (open bars). These data show that the above dose of IFN $\gamma$  can be detected in serum for about two hours following oral administration.

### Example 3

#### Prevention of Chronic Relapse of Experimental Allergic Encephalomyelitis by Orally-Administered OvIFN $\gamma$

10 The ability of OvIFN $\gamma$  to prevent paralysis was examined using a chronic-relapsing model of EAE, in which SJL mice immunized with MBP develop a chronic form of the disease where the appearance of symptoms occurs in a relapsing-remitting manner (Zamvil and Steinman, 1990).

EAE was induced in SJL mice essentially as described above. The mice were treated with  $10^5$  U of OvIFN $\gamma$  by either i.p. injection or oral feeding on the day of immunization (day 0) and every 48 hours thereafter for the duration of the experiment. As presented in Figure 2A, SJL mice which were immunized with MBP but did not receive OvIFN $\gamma$  treatment developed chronic relapsing paralysis with a 5/5 incidence of disease, with a peak mean severity of  $\sim 2.5$  occurring 14 days after the start of the experiment. In contrast, treatment with OvIFN $\gamma$  by either i.p. injection or oral feeding (Figures 2B and 2C, respectively) resulted in protection from EAE. 15 Incidence of disease in both OvIFN $\gamma$  treatment groups was reduced to 1/5 animals, with a mean severity of  $\sim 1.0$ . These data indicate that oral administration of IFN $\gamma$  can block the development of chronic relapsing EAE, and suggest that orally-administered IFN $\gamma$  may be as effective as i.p. injection when the IFN $\gamma$  is fed about every 48 hours over an extended period of time.

### Example 4

#### Histological Analysis

25 Histological analyses were performed to determine the extent of lymphocyte infiltration into the CNS of MBP-immunized mice treated with OvIFN $\gamma$  by oral and i.p. routes.

Mice were perfused with 4% paraformaldehyde, vertebral columns were removed and treated with formalin for 2 to 3 days. Spinal cords were dissected out and soaked in 0.5 % sucrose overnight at 4°C. Spinal cord sections were embedded and sections cut in a microtome. Sections were fixed to slides in 4 % paraformaldehyde and stained with cresyl violet for visualization of inflammatory infiltrates. 30

The results are shown in Figures 3A, 3B and 3C at a final magnification of 222 $\times$ . Lymphocytic lesions were present in control spinal cord white matter (Fig. 3A). In contrast, no lymphocytic infiltrates were detected in mice treated with OvIFN $\gamma$  by i.p. injection (Fig. 3B) or 35

oral feeding (Fig. 3C). These data suggest that the protective effect of IFN $\gamma$  is associated with inhibition of lymphocyte infiltration of the CNS.

#### Example 5

##### Induction of IL10 by Treatment with OvIFN $\gamma$

5 During the course of OvIFN $\gamma$  treatment of SJL for prevention of chronic relapsing EAE, mice were bled and sera were examined for the presence of interleukin 10 (IL10). Sera from mice which received either a single IFN $\gamma$  ( $10^5$  U) treatment (by i.p. injection or oral feeding), prolonged IFN $\gamma$  ( $10^5$  U) treatment (by i.p. injection or oral treatment for greater than 20 days) or no treatment were examined for IL10 by enzyme-linked immunosorbent assay (ELISA) using IL10  
10 ELISA kits (Genzyme, Cambridge, MA) following the manufacturer's instructions. All sera samples were tested in duplicate.

No IL10 was detected in control mice or in mice which received a single treatment of OvIFN $\gamma$  by either i.p. injection or oral feeding. In contrast, SJL mice which received OvIFN $\gamma$  by either i.p. injection or oral feeding every 48 hours for greater than 20 days had detectable  
15 levels of IL10 in their sera (Figure 4). These data suggest that IFN $\gamma$ -induced production of IL10 may be a contributing mechanism by which OvIFN $\gamma$  prevents development of EAE.

#### Example 6

##### Cessation of Treatment with OvIFN $\gamma$ Results in Relapsing Paralysis

SJL mice which were protected from EAE by OvIFN $\gamma$  treatment via i.p. injection or oral  
20 feeding (every 48 hours) were followed for 58 days, during which time no disease development was observed. Treatment with OvIFN $\gamma$  was then removed and the mice were observed for an additional 22 days for symptoms of disease.

The results are shown in Figure 5. IFN $\gamma$  treatment is denoted as plus signs and removal of IFN $\gamma$  treatment is denoted as minus signs beneath the graph. Disease incidence in each treatment  
25 group was as follows: PBS control=3/4 (square); i.p. injection=3/3 (triangle); oral feeding=3/4 (circle).

Both groups of mice which had previously been protected from EAE by OvIFN $\gamma$  treatment developed signs of paralysis 6 to 12 days after removal of the OvIFN $\gamma$  treatment. These data indicate that ongoing administration of IFN $\gamma$ , by either i.p. injection or oral feeding, is desirable  
30 for continued protection from EAE in the chronic-relapsing model of EAE.

Example 7Oral Administration of OvIFN $\gamma$  Reduces Anti-OvIFN $\gamma$  Antibody Response

After removal of OvIFN $\gamma$  treatment in the experiments described in Example 6, above, mice from each treatment group were bled and sera were examined for the presence of anti-OvIFN $\gamma$  antibodies (Ab).

The antigen, OvIFN $\gamma$ , was adsorbed to the flat bottoms of plastic tissue culture wells overnight at a concentration of 600 ng/well, and subsequently evaporated to dryness. The plates were treated with 5% milk (Carnation) in PBS for 2 hours in order to block nonspecific binding and then washed 3 times with PBS containing 0.05% Tween 20. Various dilutions of sera from mice which were untreated, OvIFN $\gamma$  treated by i.p. injection and OvIFN $\gamma$  treated by oral feeding were added and incubated for 3 hours. Binding was assessed with goat anti-mouse immunoglobulin coupled to horseradish peroxidase. Color development was monitored at 492 nm in an ELISA plate reader (Bio-Rad, Richmond, CA) after o-phenylenediamine and H<sub>2</sub>O<sub>2</sub> were added and the reaction terminated with 2M H<sub>2</sub>SO<sub>4</sub>.

Exemplary results are shown in Figure 6. Sera from untreated, OvIFN $\gamma$  treated-i.p. injected and OvIFN $\gamma$  treated-orally fed (2 mice/group) were examined by ELISA using multiple dilutions, including 1:30 (open bars) and 1:120 (filled bars). Mice which received OvIFN $\gamma$  by oral feeding exhibited minimal Ab levels while mice which received OvIFN $\gamma$  by i.p. injection exhibited elevated levels of anti-OvIFN $\gamma$  Ab. As expected, mice which received no OvIFN $\gamma$  treatment displayed no anti-OvIFN $\gamma$  Ab.

Sera were also examined for their ability to neutralize OvIFN $\gamma$  antiviral activity on MDBK cells as described above. The results are shown in Table 4, below. None of the sera from either i.p. injected or orally fed mice possessed neutralizing activity. These data suggest that oral treatment with IFN $\gamma$  circumvents the Ab response directed against OvIFN $\gamma$  protein observed in i.p. injection-treated individuals, and that neither treatment typically results in the generation of neutralizing antibodies.

Table 4

Sera from Mice Treated with OvIFN $\gamma$  by i.p. Injection  
or Oral Feeding do Not Possess Neutralizing Activity

5

500 U/ML OF OVIFNT COCULTURED WITH SERA FROM:	OVIFNT TITER (U/ML)
untreated	500
i.p. injected	500
orally fed	500
HL127	<50

10

While the invention has been described with reference to specific methods and embodiments, it is appreciated that various modifications and changes may be made without departing from the invention.

## SEQUENCE LISTING

- (1) GENERAL INFORMATION:
- (i) APPLICANT: UNIVERSITY OF FLORIDA
- 5 (ii) TITLE OF INVENTION: Orally-Administered Interferon-Tau  
Compositions and Methods
- (iii) NUMBER OF SEQUENCES: 6
- (iv) CORRESPONDENCE ADDRESS:
- 10 (A) ADDRESSEE: Dehlinger & Associates  
(B) STREET: 350 Cambridge Ave., Suite 250  
(C) CITY: Palo Alto  
(D) STATE: CA  
(E) COUNTRY: USA  
15 (F) ZIP: 94306
- (v) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
20 (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
- (A) APPLICATION NUMBER:  
(B) FILING DATE: 12-MAR-1997  
(C) CLASSIFICATION:
- 25 (vii) PRIOR APPLICATION DATA:
- (A) APPLICATION NUMBER: US 08/616,904  
(B) FILING DATE: 15-MAR-1996
- (viii) ATTORNEY/AGENT INFORMATION:
- 30 (A) NAME: Sholtz, Charles K.  
(B) REGISTRATION NUMBER: 38,615  
(C) REFERENCE/DOCKET NUMBER: 5600-0003.41
- (ix) TELECOMMUNICATION INFORMATION:
- (A) TELEPHONE: 415-324-0880  
(B) TELEFAX: 415-324-0960
- 35 (2) INFORMATION FOR SEQ ID NO:1:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 516 base pairs  
(B) TYPE: nucleic acid  
40 (C) STRANDEDNESS: double  
(D) TOPOLOGY: circular
- (ii) MOLECULE TYPE: DNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
- 45 (A) ORGANISM: Ovis aries  
(B) STRAIN: Domestic  
(D) DEVELOPMENTAL STAGE: Blastula (blastocyst)  
(F) TISSUE TYPE: Trophectoderm  
(G) CELL TYPE: Mononuclear trophectoderm cells
- 50 (vii) IMMEDIATE SOURCE:
- (B) CLONE: oTP-1a



(viii) POSITION IN GENOME:  
(C) UNITS: bp

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..516

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Ott, Troy L  
Van Heeke, Gino  
Johnson, Howard M  
Bazer, Fuller W

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(C) JOURNAL: J. Interferon Res.

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(K) RELEVANT RESIDUES IN SEQ ID NO:1: FROM 1 TO 516

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CTG CTG GAC CGT ATG AAT CGA TTG TCT CCG CAC AGC TGC CTG CAA GAC      96
Leu Leu Asp Arg Met Asn Arg Leu Ser Pro His Ser Cys Leu Gln Asp      30
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CGG AAA GAC TTC GGT CTG CCG CAG GAA ATG GTT GAA GGT GAC CAA CTG      144
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CAA AAA GAC CAA GCT TTC CCG GTA CTG TAT GAA ATG CTG CAG CAG TCT      192
Gln Lys Asp Gln Ala Phe Pro Val Leu Tyr Glu Met Leu Gln Gln Ser      60
  50                               55

TTC AAC CTG TTC TAC ACT GAA CAT TCT TCG GCC GCT TGG GAC ACT ACT      240
Phe Asn Leu Phe Tyr Thr Glu His Ser Ser Ala Ala Trp Asp Thr Thr      80
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CTT CTA GAA CAA CTG TGC ACT GGT CTG CAA CAG CAA CTG GAC CAT CTG      288
Leu Leu Glu Gln Leu Cys Thr Gly Leu Gln Gln Gln Leu Asp His Leu      95
  85                               90

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  100                              105

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Asn Met Asp Pro Ile Val Thr Val Lys Lys Tyr Phe Gln Gly Ile Tyr      125
  115                              120

GAC TAC CTG CAG GAA AAA GGT TAC TCT GAC TGC GCT TGG GAA ATC GTA      432
Asp Tyr Leu Gln Glu Lys Gly Tyr Ser Asp Cys Ala Trp Glu Ile Val      140
  130                              135

CGC GTT GAA ATG ATG CGG GCC CTG ACT GTG TCG ACT ACT CTG CAA AAA      480
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CGG TTA ACT AAA ATG GGT GGT GAC CTG AAT TCT CCG      516

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Arg Leu Thr Lys Met Gly Gly Asp Leu Asn Ser Pro  
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(2) INFORMATION FOR SEQ ID NO:2:

- 5 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 172 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- 10 (vi) ORIGINAL SOURCE:  
(C) INDIVIDUAL ISOLATE: amino acid sequence of a mature  
OvIFNtau protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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1 5 10 15

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35 40 45

20 Gln Lys Asp Gln Ala Phe Pro Val Leu Tyr Glu Met Leu Gln Gln Ser  
50 55 60

Phe Asn Leu Phe Tyr Thr Glu His Ser Ser Ala Ala Trp Asp Thr Thr  
65 70 75 80

Leu Leu Glu Gln Leu Cys Thr Gly Leu Gln Gln Gln Leu Asp His Leu  
85 90 95

25 Asp Thr Cys Arg Gly Gln Val Met Gly Glu Glu Asp Ser Glu Leu Gly  
100 105 110

Asn Met Asp Pro Ile Val Thr Val Lys Lys Tyr Phe Gln Gly Ile Tyr  
115 120 125

30 Asp Tyr Leu Gln Glu Lys Gly Tyr Ser Asp Cys Ala Trp Glu Ile Val  
130 135 140

Arg Val Glu Met Met Arg Ala Leu Thr Val Ser Thr Thr Leu Gln Lys  
145 150 155 160

Arg Leu Thr Lys Met Gly Gly Asp Leu Asn Ser Pro  
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35 (2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 516 base pairs  
(B) TYPE: nucleic acid  
40 (C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:  
(C) INDIVIDUAL ISOLATE: synthetic nucleotide sequence encoding  
a mature human interferon-tau protein, HuIFNtau1.

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TGTGACTTGT CTCAAACCA CGTTTTGGTT GGTAGAAAGA ACTTAAGACT ACTAGACGAA  
 60  
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 5 GAAATGGTTG AAGGTGGCCA ACTACAAGAA GCTCAAGCGA TATCTGTTTT GCACGAAATG 180  
 TTGCAACAAA GCTTCAACTT GTTCCACACC GAACACTCTT CGGCCGCTTG GGACACCACC 240  
 TTGTTGGAAC AGCTCAGAAC CGGTTTGCAC CAACAATTGG ACAACTTGGA TGCATGTTTG 300  
 GGTCAAGTTA TGGGTGAAGA AGACTCTGCT CTCGGGAGAA CCGGTCCAAC GCTAGCTTTG 360  
 AAGAGATACT TCCAAGGTAT CCACGTTTAC TTGAAGGAAA AGGGTTACTC TGACTGTGCT 420  
 10 TGGGAAACCG TCGTCTAGA AATCATGCGT AGCTTCTCTT CTTTGATCAG CTTGCAAGAA 480  
 AGATTACGTA TGATGGACGG TGACTTGTCG AGCCCA 516

## (2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 172 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 15  
 (ii) MOLECULE TYPE: protein  
 (vi) ORIGINAL SOURCE:  
 20 (C) INDIVIDUAL ISOLATE: amino acid sequence for a mature  
 HuIFNtau protein, HuIFNtau1.

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Cys Asp Leu Ser Gln Asn His Val Leu Val Gly Arg Lys Asn Leu Arg  
 1 5 10 15  
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 20 25 30  
 Arg Lys Asp Phe Ala Leu Pro Gln Glu Met Val Glu Gly Gly Gln Leu  
 35 40 45  
 30 Gln Glu Ala Gln Ala Ile Ser Val Leu His Glu Met Leu Gln Gln Ser  
 50 55 60  
 Phe Asn Leu Phe His Thr Glu His Ser Ser Ala Ala Trp Asp Thr Thr  
 65 70 75 80  
 Leu Leu Glu Gln Leu Arg Thr Gly Leu His Gln Gln Leu Asp Asn Leu  
 85 90 95  
 35 Asp Ala Cys Leu Gly Gln Val Met Gly Glu Glu Asp Ser Ala Leu Gly  
 100 105 110  
 Arg Thr Gly Pro Thr Leu Ala Leu Lys Arg Tyr Phe Gln Gly Ile His  
 115 120 125  
 40 Val Tyr Leu Lys Glu Lys Gly Tyr Ser Asp Cys Ala Trp Glu Thr Val  
 130 135 140  
 Arg Leu Glu Ile Met Arg Ser Phe Ser Ser Leu Ile Ser Leu Gln Glu  
 145 150 155 160

Arg Leu Arg Met Met Asp Gly Asp Leu Ser Ser Pro  
165 170

## (2) INFORMATION FOR SEQ ID NO:5:

- 5 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 516 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- 10 (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:  
 (C) INDIVIDUAL ISOLATE: HuIFNtau3, mature no leader sequence
- 15 (ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 1..516
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
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1 5 10 15
- CTC CTG GGC CAA ATG AGG AGA CTC TCC CTT CGC TTC TGT CTG CAG GAC  
96  
 25 Leu Leu Gly Gln Met Arg Arg Leu Ser Leu Arg Phe Cys Leu Gln Asp  
20 25 30
- AGA AAA GAC TTC GCT TTC CCC CAG GAG ATG GTG GAG GGT GGC CAG CTC 144  
 Arg Lys Asp Phe Ala Phe Pro Gln Glu Met Val Glu Gly Gly Gln Leu  
35 40 45
- CAG GAG GCC CAG GCC ATC TCT GTG CTC CAC GAG ATG CTC CAG CAG AGC 192  
 30 Gln Glu Ala Gln Ala Ile Ser Val Leu His Glu Met Leu Gln Gln Ser  
50 55 60
- TTC AAC CTC TTC CAC ACA GAG CAC TCC TCT GCT GCC TGG GAC ACC ACC 240  
 Phe Asn Leu Phe His Thr Glu His Ser Ser Ala Ala Trp Asp Thr Thr  
65 70 75 80
- 35 CTC CTG GAG CAG CTC CGC ACT GGA CTC CAT CAG CAG CTG GAT GAC CTG 288  
 Leu Leu Glu Gln Leu Arg Thr Gly Leu His Gln Gln Leu Asp Asp Leu  
85 90 95
- GAT GCC TGC CTG GGG CAG GTG ACG GGA GAG GAA GAC TCT GCC CTG GGA 336  
 40 Asp Ala Cys Leu Gly Gln Val Thr Gly Glu Glu Asp Ser Ala Leu Gly  
100 105 110
- AGA ACG GGC CCC ACC CTG GCC ATG AAG AGG TAT TTC CAG GGC ATC CAT 384  
 Arg Thr Gly Pro Thr Leu Ala Met Lys Arg Tyr Phe Gln Gly Ile His  
115 120 125
- 45 GTC TAC CTG AAA GAG AAG GGA TAT AGT GAC TGC GCC TGG GAA ATT GTC 432  
 Val Tyr Leu Lys Glu Lys Gly Tyr Ser Asp Cys Ala Trp Glu Ile Val  
130 135 140
- AGA CTG GAA ATC ATG AGA TCC TTG TCT TCA TCA ACC AGC TTG CAC AAA 480  
 Arg Leu Glu Ile Met Arg Ser Leu Ser Ser Ser Thr Ser Leu His Lys

145	150	155	160	
AGG TTA AGA ATG ATG GAT GGA GAC CTG AGC TCA CCT				
Arg	Leu	Arg	Met	Asp
				Gly
				Asp
				Leu
				Ser
				Ser
				Pro
	165		170	

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5 (2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 172 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

	Cys	Asp	Leu	Ser	Gln	Asn	His	Val	Leu	Val	Gly	Ser	Gln	Asn	Leu	Arg
	1				5					10					15	
	Leu	Leu	Gly	Gln	Met	Arg	Arg	Leu	Ser	Leu	Arg	Phe	Cys	Leu	Gln	Asp
15				20					25					30		
	Arg	Lys	Asp	Phe	Ala	Phe	Pro	Gln	Glu	Met	Val	Glu	Gly	Gly	Gln	Leu
			35					40					45			
	Gln	Glu	Ala	Gln	Ala	Ile	Ser	Val	Leu	His	Glu	Met	Leu	Gln	Gln	Ser
		50					55					60				
20	Phe	Asn	Leu	Phe	His	Thr	Glu	His	Ser	Ser	Ala	Ala	Trp	Asp	Thr	Thr
	65					70					75					80
	Leu	Leu	Glu	Gln	Leu	Arg	Thr	Gly	Leu	His	Gln	Gln	Leu	Asp	Asp	Leu
					85					90					95	
	Asp	Ala	Cys	Leu	Gly	Gln	Val	Thr	Gly	Glu	Glu	Asp	Ser	Ala	Leu	Gly
25				100					105					110		
	Arg	Thr	Gly	Pro	Thr	Leu	Ala	Met	Lys	Arg	Tyr	Phe	Gln	Gly	Ile	His
			115					120					125			
	Val	Tyr	Leu	Lys	Glu	Lys	Gly	Tyr	Ser	Asp	Cys	Ala	Trp	Glu	Ile	Val
		130					135					140				
30	Arg	Leu	Glu	Ile	Met	Arg	Ser	Leu	Ser	Ser	Ser	Thr	Ser	Leu	His	Lys
	145				150					155						160
	Arg	Leu	Arg	Met	Met	Asp	Gly	Asp	Leu	Ser	Ser	Pro				
					165					170						

## IT IS CLAIMED:

1. In a method of treating a disease condition in a mammal responsive to treatment by interferon-tau (IFN $\tau$ ), an improvement comprising orally administering a therapeutically-effective amount of IFN $\tau$ .
- 5 2. The method of claim 1, wherein IFN $\tau$  is orally-administered at a dosage of between about  $1 \times 10^5$  and about  $1 \times 10^8$  units per day.
3. The method of claim 2, wherein IFN $\tau$  is orally-administered at a dosage of between about  $1 \times 10^6$  and about  $1 \times 10^7$  units per day.
4. The method of claim 1, wherein the orally-administered IFN $\tau$  is ovine IFN $\tau$  (OvIFN $\tau$ ).
- 10 5. The method of claim 1, wherein said OvIFN $\tau$  has the sequence represented as SEQ ID NO:2.
6. The method of claim 1, wherein the orally-administered IFN $\tau$  is human IFN $\tau$  (HuIFN $\tau$ ).
7. The method of claim 1, wherein said HuIFN $\tau$  has the sequence represented as SEQ ID NO:4.
8. The method of claim 1, wherein said mammal is a human.
9. The method of claim 1, wherein said mammal is a dog.
- 15 10. The method of claim 1, wherein said disease condition is an immune system disorder.
11. The method of claim 10, wherein said disease condition is an autoimmune disorder.
12. The method of claim 11, wherein said autoimmune disorder is selected from the group consisting of multiple sclerosis, type I (insulin dependent) diabetes mellitus, lupus erythematosus, amyotrophic lateral sclerosis, Crohn's disease, rheumatoid arthritis, stomatitis, asthma, allergies  
20 and psoriasis.

13. The method of claim 12, wherein said autoimmune disorder is selected from the group consisting of multiple sclerosis, rheumatoid arthritis, lupus erythematosus and type I diabetes mellitus.
14. The method of claim 13, wherein said autoimmune disorder is multiple sclerosis.
- 5 15. A method of treating an autoimmune disorder in a subject, comprising orally administering a therapeutically-effective amount of interferon-tau (IFN $\tau$ ) to said subject.
16. The method of claim 15, wherein said autoimmune disorder is selected from the group consisting of multiple sclerosis, rheumatoid arthritis, lupus erythematosus and type I diabetes mellitus.
- 10 17. A method of claim 16, wherein said autoimmune disorder is multiple sclerosis.
18. The method of claim 15, wherein IFN $\tau$  is orally-administered at a dosage of between about  $1 \times 10^5$  and about  $1 \times 10^8$  units per day.
19. The method of claim 18, wherein IFN $\tau$  is orally-administered at a dosage of between about  $1 \times 10^6$  and about  $1 \times 10^7$  units per day.

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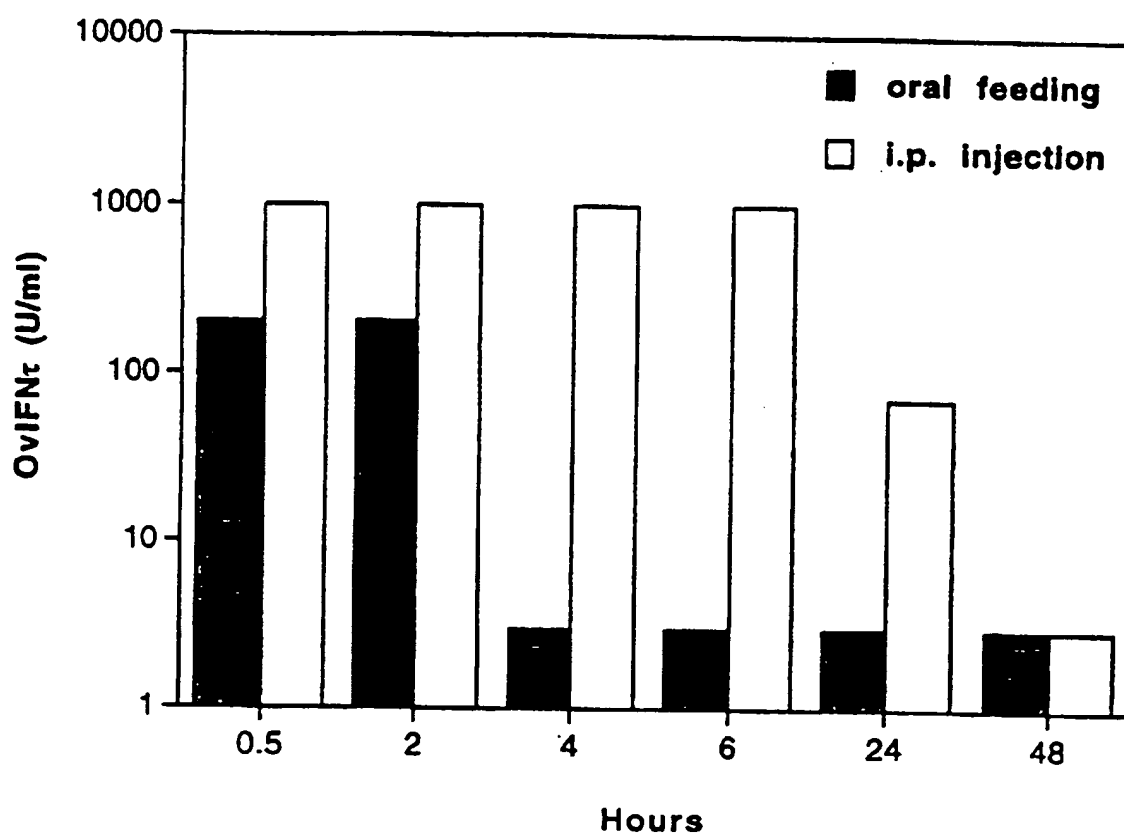


Fig. 1



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Fig. 2A

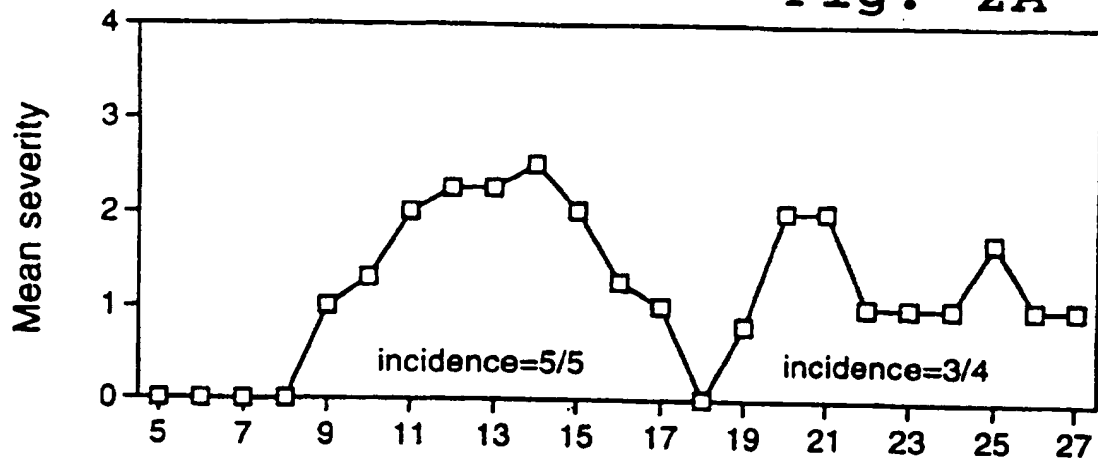
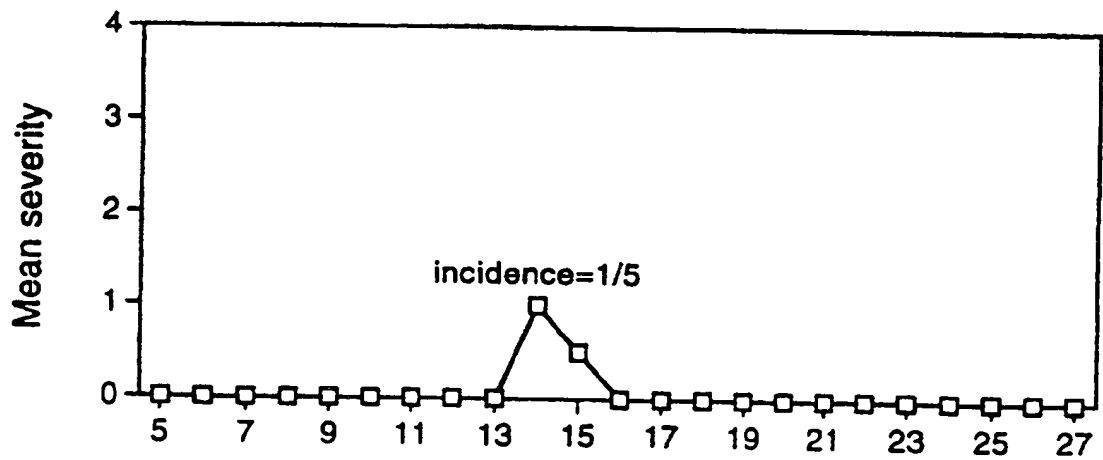
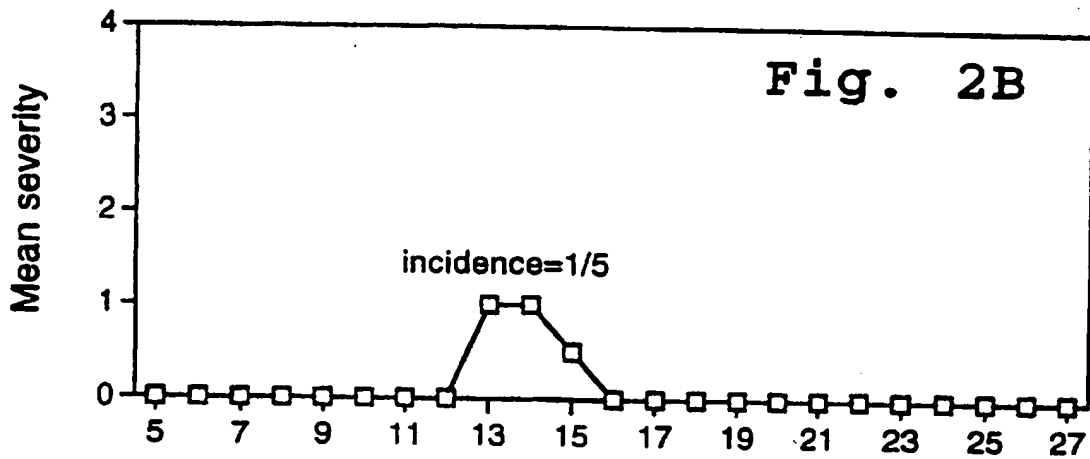


Fig. 2B



Days

Fig. 2C

Fig. 3A

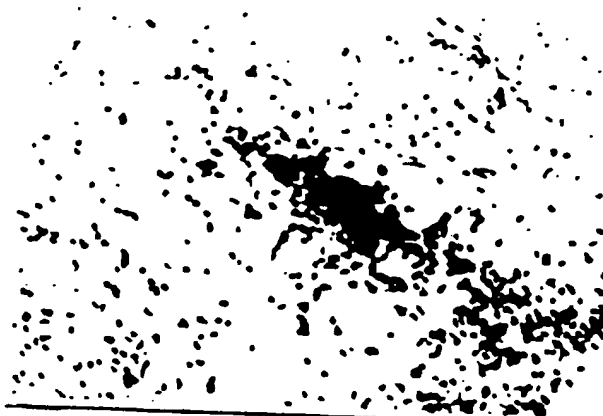


Fig. 3B

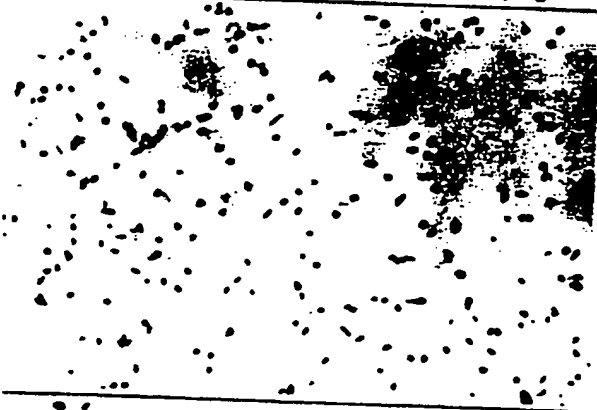
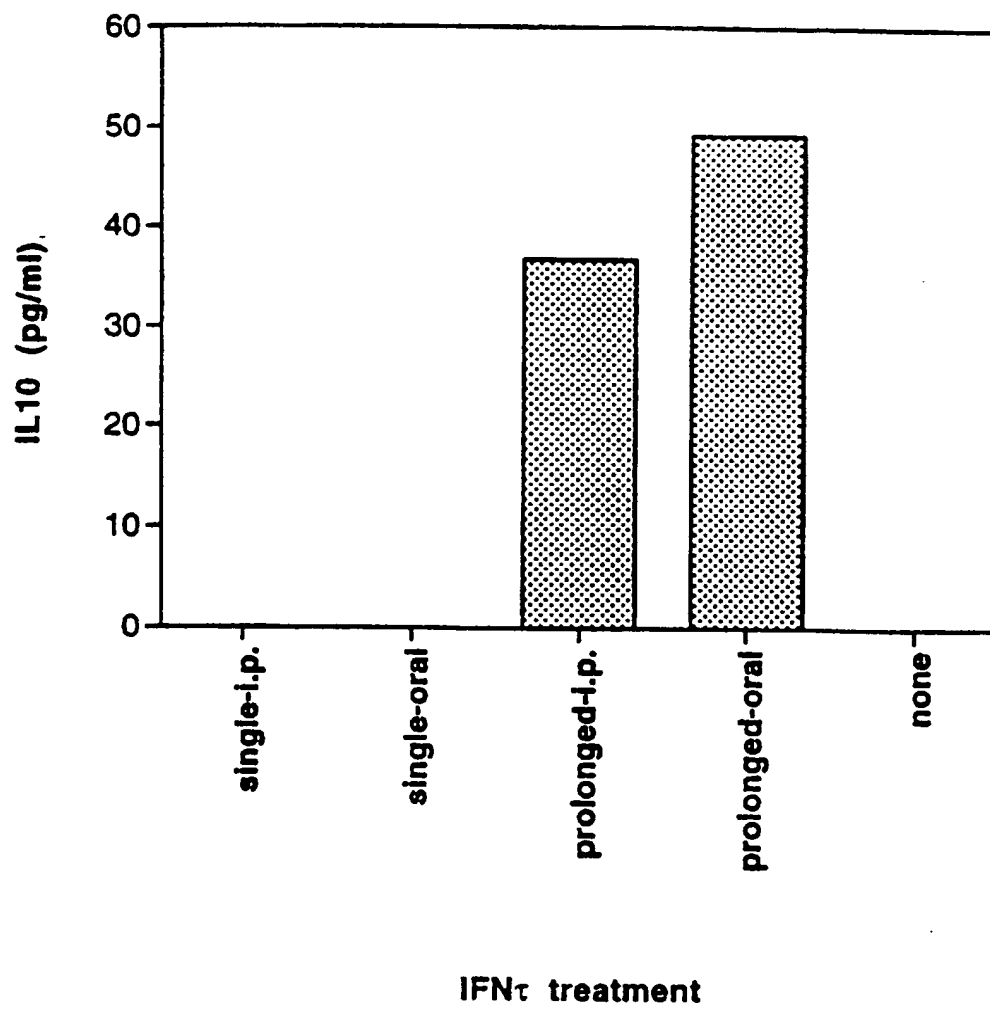
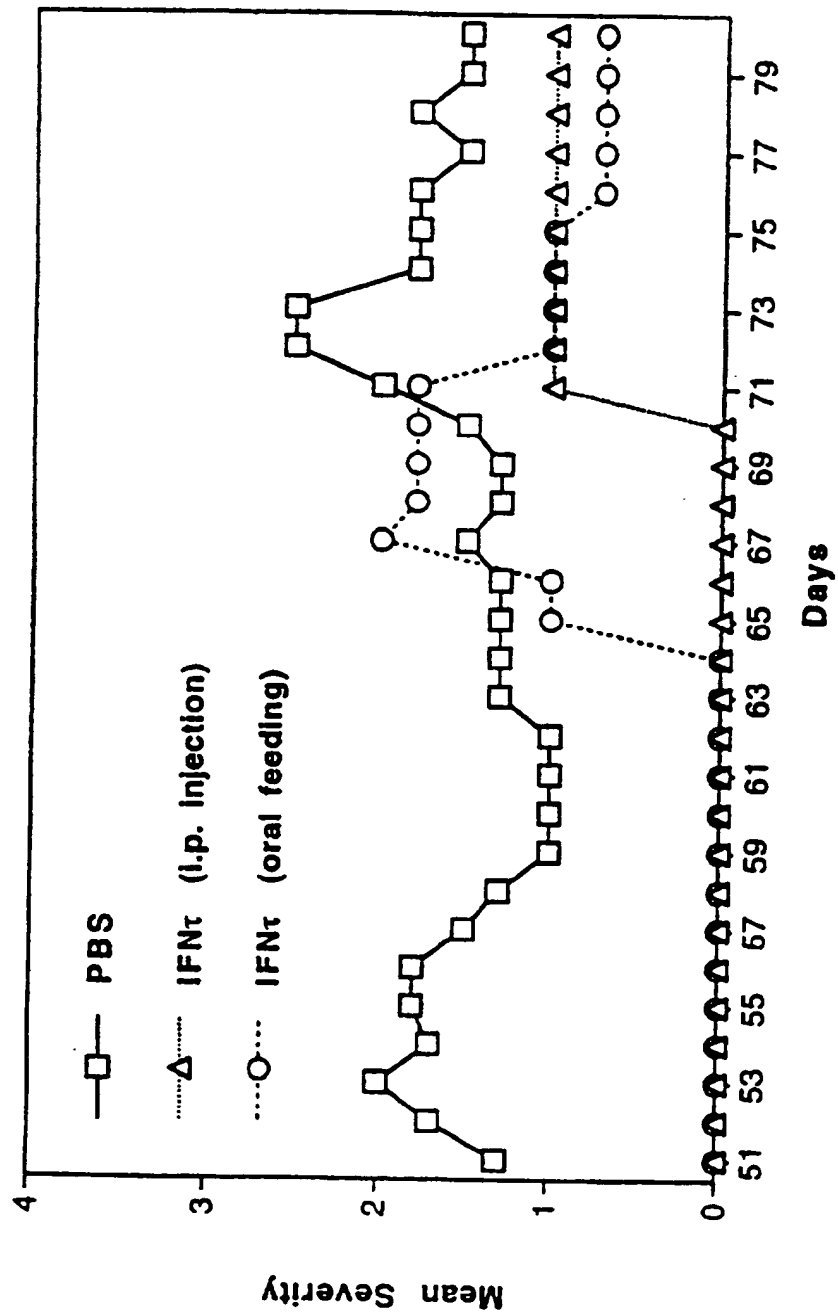


Fig. 3C



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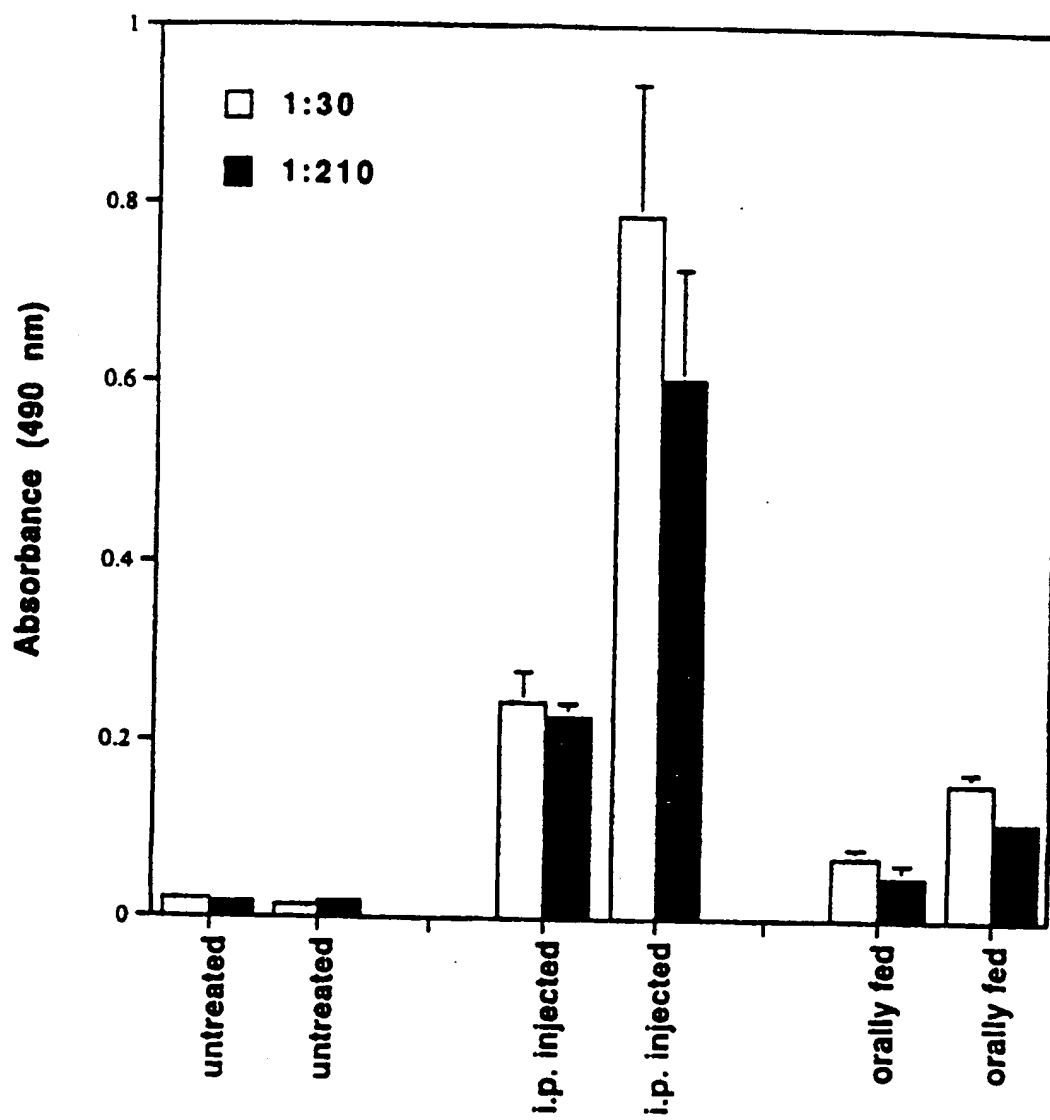
**Fig. 4**



**IFN $\tau$  treatment:**

5. உரு

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IFN̳ Treatment

Fig. 6

# INTERNATIONAL SEARCH REPORT

Internat. Application No.  
PCT/US 97/03794

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 A61K38/21

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF IMMUNOLOGY, vol. 155, no. 5, 1 September 1995, BALTIMORE US, pages 2747-2753, XP002010954 J.M. SOOS ET AL.: "THE IFN PREGNANCY RECOGNITION HORMONE IFN-TAU BLOCKS BOTH DEVELOPMENT AND SUPERANTIGEN REACTIVATION OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS WITHOUT ASSOCIATED TOXICITY." see page 2751, right-hand column, line 27 - page 2752, left-hand column, paragraph 2 --- -/--	1,4-17

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

\* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- \*&\* document member of the same patent family

Date of the actual completion of the international search

20 June 1997

Date of mailing of the international search report

07.07.97

Name and mailing address of the ISA

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Fax (+31-70) 340-3016

Authorized officer

Ryckebosch, A

# INTERNATIONAL SEARCH REPORT

Internals 1 Application No  
PCT/US 97/03794

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>FASEB JOURNAL FOR EXPERIMENTAL BIOLOGY, vol. 9, no. 4, 10 March 1995, BETHESDA, MD US, page A1025 XP002010951 J.M. SOOS ET AL.: "THE NOVEL TYPE I INTERFERON TAU PREVENTS DEVELOPMENT AND SUPERANTIGEN REACTIVATION OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN MICE WITHOUT ASSOCIATED TOXICITY." see abstract nr. 5940 ---</p>	1,4-17
X	<p>WO 90 09806 A (UNIVERSITY OF FLORIDA) 7 September 1990 see page 3, line 27 - page 4, line 3; claims 1-10; figure 1; example 8 see page 16, line 1 - line 19 ---</p>	1-9
P,X	<p>WO 96 28183 A (UNIVERSITY OF FLORIDA) 19 September 1996 see the whole document -----</p>	1-19

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 97/03794

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-19  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claim(s) 1-19  
is(are) directed to a method of treatment of the human/animal  
body, the search has been carried out and based on the alleged  
effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such  
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all  
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment  
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report  
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is  
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.



# INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern: d Application No

PCT/US 97/03794

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9009806 A	07-09-90	AU 5183490 A	26-09-90
WO 9628183 A	19-09-96	AU 5422396 A	02-10-96